THE PERMEABILITY OF RED CORPUSCLES TO VARIOUS SALTS OF GLUCONIC ACID

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA
June, 1959

ACKNOWLEDGMENTS

The writer wishes to express his deepest appreciation to the Chairman of his Supervisory Committee, Dr. William J. Husa, under whose attentive guidance this investigation was accomplished. His understanding and concern throughout have been invaluable and most inspiring.

Gratitude is extended to Dr. George E. Ryschkewitsch for his time and advice so generously given. His assistance in the explanation of certain chemical mechanisms has been especially helpful and is sincerely appreciated.

The interest shown by the other members of the writer's Supervisory Committee, Drs. Werner M. Lauter, Thomas D. Malewitz, and Randall B. Tinker, has indeed been stimulating.

The writer is indebted to both the Graduate Council and the American Foundation for Pharmaceutical Education for their encouragement and financial assistance.

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INTRODUCTION

The adjustment of solutions intended for intravenous administration to the proper tonicity is of vital concern. The value of employing the hemolytic method in the determination of i values has been pointed out by Husa and co-workers (1, 2, 3, 4, 5, 6, 7, 8). They emphasized that the osmotic effect of a substance on the erythrocyte depends not only on the concentration of the substance but also on whether or not the compound penetrates the cell membrane or affects its properties. Thus solutions calculated to be isosmotic with blood according to physicochemical data are not necessarily isotonic to the red blood cell.

Cadwallader and Husa (5) reported that zinc acetate gave unusually high hemolytic i values, much beyond those calculated from colligative property information. Their work complemented that of Hartman and Husa (4), who found similarly high hemolytic i values for zinc sulfate.

The purpose of the present work was to investigate the elements closely related to zinc in the periodic table to determine whether or not their action paralleled that of zinc. Added interest in this investigation was stimulated by the fact that these elements, i.e., copper, cobalt, iron, manganese, and zinc are so called "trace elements," essential to human nutrition. To obtain a comparative picture of the activities of these five cations, they were all employed as salts of gluconic acid. Data pertaining to their effects on the red blood cell is of momentous value since these minerals may soon be incorporated into vitamin injectables. Sodium, potassium, magnesium, and calcium gluconates were also studied and their hemolytic i values determined; these cations are normally present in large amounts in the human body, being known as "major elements," and are widely used in medicine.

REVIEW OF THE LITERATURE

Gluconic Acid

Gluconic acid is a pentahydroxycaproic acid, $CH_2OH(CHOH)_4COOH$, discovered in 1870 as a product of the oxidation of glucose with chlorine by Hlasiwetz and Haberman (9). Boutroux (10), in 1878, during his investigations of lactic acid fermentations, first isolated gluconic acid. It was not until the 1930's, however, that gluconic acid became commercially available.

At the present time, gluconic acid is produced commercially by the fermentative oxidation of the aldehyde group of glucose (11). This oxidation may also be carried out in alkaline media with the utilization of hypobromites (12), or electrolytically employing an insoluble anode in the presence of a water soluble iodide (13). Although gluconic acid may be prepared as a solid product (14), the high cost of the complex procedure has necessitated the production of a 50 per cent aqueous solution to represent the acid of commerce.

In aqueous solution gluconic acid exists in equilibrium with its corresponding gamma- and delta-lactones, the concentrations of which vary with the original gluconic acid concentration and the temperature. Upon evaporation of its aqueous solution, gluconic acid passes into commercially important d-gluconolactone (15).

Hermann (16) found that free gluconic acid was well tolerated when taken with the daily food for years by humans and animals, producing neither injury nor disturbance to the digestive organs. On the basis of studies conducted with 0.25 M. acid solutions administered intravenously, it was reported that gluconic acid was the least toxic of the following acids listed in order of decreasing toxicity: oxalic, malonic, phosphoric, tartaric, pyromucic, hydrochloric, lactic, formic, propionic, succinic, acetic, and gluconic (17). Rabbits have been treated daily with both subcutaneous and intravenous injections of gluconic acid without any indication of ill effects after one week (18). In 1940, the Committee of Foods of the American Medical Association pronounced the purified delta-lactone of gluconic acid to be safe for use in foods (19). Small proportions of gluconic acid added to fats and oils produced products which were less likely to become rancid (20).

Gluconic acid has been demonstrated to be useful in the solution of existing phosphate stones in the human kidney (16) as well as in the prevention of their formation (21). Both gluconic acid and glucurono-delta-lactone have been used effectively to acidify the urine (22, 23). It has been reported (24) that gluconic acid was preferable to mandelic acid in the treatment of pyelonephritis due to gram-negative bacilli. The therapeutic effects were reported to have been equally effective with each, however, most patients examined suffered less gastrointestinal and renal distress when treated with gluconic acid than when mandelic acid was used.

Gluconates

Since the gluconate anion is without marked pharmacological activity or toxicity, metallic gluconates may be administered as effective sources of the cation without any complications arising from the presence of the gluconate radical (25). Many metallic salts of gluconic acid have been prepared and purified, such as the gluconates of aluminum, ammonium, antimony, barium, bismuth, cadmium, calcium, cobalt, copper, iron, lead, magnesium, manganese, potassium, sodium, and zinc (26, 27, 28, 29, 30). Although most of these salts are not currently being used in medicine, the possibility of utilizing several of them for trace element administration has been suggested (25).

Gluconic acid has also been employed in the preparation of salts of drugs which are organic bases. These include brucine, choline, cinchonidine, cinchonine, ephedrine, procaine amide, quinidine, quinine, streptomycin, and strychnine gluconates (31, 32, 33). A good many of these salts have been favorably employed in medicine with reports of less toxicity than when the organic bases were administered per se or combined with inorganic anions. For instance, ephedrine sulfate was reported to be more irritating than ephedrine gluconate (34). Procaine amide gluconate was injected intramuscularly with little irritation and only mild toxic effects whereas similar injections with procaine amide base usually resulted in hypotension and an occasional fatality (32).

Trivalent sodium antimony gluconate has been employed in intensive treatment of urinary schistosomiasis (35). Prescott et al. (11) reported that a preservative for human and animal blood used for transfusions has been prepared from gluconic acid in combination with tertiary aliphatic amines.

The literature is replete with uses for calcium gluconate, varying from the counteraction of general and local sensitivities to streptomycin

(36) to the dramatic elimination of withdrawal symptoms associated with drug addiction (37). The salt has been used by intravenous administration to relieve patients suffering from intestinal tuberculosis (38). It has been used in combination with ethyl morrhuate in the treatment of rheumatoid arthritis (39) as well as with a salt of d-tubocurarine in the treatment of spastic children (40). Calcium gluconate in combination with pitocin has been used in over 200 inductions of labor with safety and efficacy (41).

Although there is evidence of minimal toxicity with sodium gluconate (42), the chemical has been used very little in medicine to date. Serum levels of chlortetracycline were reported to have been increased two to three fold by simultaneous administration of sodium gluconate (43).

In medicine, potassium gluconate has found limited use in cases of hypopotassemia, infantile diarrhea, and post surgical operations (44). It has also been successfully employed, orally (45) and parenterally (46), in the treatment of allergic individuals, and has been found to be much better tolerated than potassium chloride.

Magnesium gluconate is well tolerated by man and animal when administered orally or parenterally (47). It has been successfully employed in cases of dysmenorrhea (48) and in certain cases of hyperirritability heretofor unbenefited by other known therapy (46).

Ammonium gluconate has been injected intravenously into a cat with no appreciable sustained injury in doses equivalent to an injection of 30 Gm. for a man on the basis of average body weight (49). It was employed in a study of the lowering of the pH value of urine, with results indicating its effectiveness in 10 Gm. doses.

Chelating Ability

The term "chelate" was proposed in 1925 by Morgan et al. (50) to designate those cyclic structures arising from the union of metallic atoms with organic and inorganic molecules. Diehl (51), in 1937, classified compounds capable of forming chelates with metallic cations. He distinguished chemical groups that unite with metals by the replacement of a hydrogen atom, as carboxyl and enolic hydroxyl groups, from those that combine with metals by simple coordination or addition without the replacement of a hydrogen, as carbonyl and alcoholic groups.

In general, the ability of the sugar acids to form water soluble complexes with metallic ions arises from the capacity of their carboxyl and hydroxyl groups to bind these cations in ring form by means of coordinate and covalent bonds (52). The chelating ability of the sugar acids has been found to be, on the average, more pronounced in alkaline solution than in either neutral or acid media due to increased ionization of the hydrogens of the hydroxyl groups.

Gluconic acid and its derivatives are capable of forming water soluble chelates with certain metallic ions. Although sodium gluconate and neutralized gluconic acid are, in themselves, relatively ineffective sequestrants for calcium, they have been shown to be superior to sodium citrate, sodium tetraphosphate and the tetrasodium salt of ethylenediamine tetraacetic acid, when in strongly alkaline solutions. Sodium gluconate was found to be superior to the three aforementioned sequestrants in complexing iron, over a range of pH 6 through pH 11. This gluconate was inferior, however, in the chelation of aluminum, cobalt, manganese, and zinc (11).

Recently, Pecsok and Juvet (53) showed that gluconic acid formed very strong complexes with most of the transition elements, contrary to an earlier opinion of other investigators (54). Pecsok and Juvet (53, 55) studied copper and lead gluconate systems polarographically, spectrophotometrically, potentiometrically, and amperometrically in order to postulate possible chelate ring structures for these materials and to determine equilibrium constants at various ionic strengths. Pecsok and Sandera (27, 28) conducted similar experiments for cadmium and iron(III) gluconates.

Mellor and Maley (56, 57) have observed that the stability of some bivalent metal complexes decreased in the following order: palladium, copper, nickel, lead, cobalt, zinc, cadmium, iron, manganese, and magnesium. Palladium and copper, capable of forming dsp^2 square planar bonds, formed the strongest chelates. The stability of the complexes appeared to decrease with increasing basicity of the metal, exemplified by the weak chelates formed with magnesium.

Trace Element Considerations

During the past century, and especially within the last thirty years, a steadily increasing number of elements have been found to be invariably present in living tissue. Definite biological associations have been discovered for certain elements present in very minute amounts, relative to the quantities of the main constituents in the tissues. These elements have been termed "trace elements."

Dutoit and Zbinden (58), in 1929, examined human blood spectrographically and reported that aluminum, calcium, iron, magnesium, manganese, phsophorus, potassium, silicon, silver, titanium, and zinc were present in all samples studied and that chromium, cobalt, germanium, lead, nickel, strontium, and tin were manifest in many of them. Today, further elements have been added to the growing list and within this group are a limited number of elements for which essential physiological roles have been unequivocally established. These are cobalt, copper, iodine, iron, manganese, and zinc. Other elements as barium, fluorine, molybdenum, and strontium are gradually acquiring justification for inclusion in this group of essential elements (59).

Since radioactive isotopes of these elements can be administered in small physiological doses, and yet measured accurately, the study of these and other elements has been greatly facilitated and simplified (60).

Iron

Menghini (61) was influential in placing the medicinal use of iron on a sound basis through his discovery, in 1746, that iron is a characteristic and essential constituent of blood. In 1886, Zinoffsky (62) showed that pure crystals of horse hemoglobin contained 0.335 per cent of iron. Many iron containing enzymes have been recognized, including two more recently discovered flavoprotein enzymes, DPNH cytochrome reductase (63) and xanthine oxidase (64).

Iron occurs in the red blood cells as hemoglobin. All mammalian hemoglobins are composed of four ferrous iron porphyrins (hemes) attached to one globin. Hemoglobin reversibly forms a red molecular compound with oxygen that exhibits a definite absorption spectrum. Union between the heme and the globin molecules is believed to be accomplished through electrostatic attraction between ionized propionic acid groups and surface basic groups of heme, and by coordination of the iron with a nitrogen-containing group of the globin, presumed to be the imidazole group of histidine (65).

Iron in the serum does not generally occur in the free state, but rather is completely bound to a specific protein, known as transferrin (66) or siderophilin (67), the sole function of which appears to be the transportation of iron. It has been suggested (68) that a small amount of iron exists in the ionic form in equilibrium with siderophilin and accounts for the iron transfer across membranes, whereas some seem to think that a more complex enzymatic factor involving ferritin accounts for the transfer (69).

Since the iron binding property of siderophilin is specific and since iron cannot exist in the serum in the free state for an extended period of time, the capacity of serum to transport iron is believed to be governed by the siderophilin content. Normally, about one-third of the siderophilin is actively transporting iron, with the remaining siderophilin comprising the latent iron-binding capacity of the serum. An intravenous injection of iron produces an increase in the proportion of iron-saturated siderophilin, with any excess of iron leaving the blood stream and entering the tissues (65).

For many years investigators have searched for an iron compound suitable for parenteral administration (70). As early as 1898, iron citrate and iron propionate were injected subcutaneously in the treatment of chlorosis (71). Physicians administering iron citrate and iron cacodylate were concerned with the pain following their injection (72, 73). Heath, et al., (74) conducted the first careful study of the utilization of iron injections and concluded that "except in rare instances, the administration of iron by the parenteral route should be avoided."

In 1936, Heilmeyer (75) injected iron ascorbate intravenously with encouraging results. In the following year, iron and ammonium citrate (76) and ferrous gluconate (77) were administered parenterally. Ferrous gluconate was reported to be void of protein precipitating action as well as causing no local or general reactions on injection into humans.

In 1945, the Bulletin of the National Formulary Committee (78) stated that intramuscular injection of iron was the only permissible parenteral route. It was further contended that since no salt of iron with protein precipitating properties should be used, the choice of iron preparations was limited to two, green iron and ammonium citrate and ferrous gluconate. The latter, being free from irritation on injection, was mentioned as the drug of choice.

Massive doses of iron as colloidal ferric hydroxide and as colloidal ferric oxide were administered intravenously in 1946 with good reticulocyte development (79). In the following year, Nissim (80) discovered that relatively large amounts of saccharated iron oxide could be tolerated intravenously. Several years later, however, a fatality was reported after an injection of saccharated iron oxide (81).

In 1954, an iron-dextran compound containing 5 per cent of iron was described (82). It was reported to be extremely stable in vivo and in vitro, with low toxicity and well tolerated parenterally while stimulating erythropoiesis.

Cobalt

The occurrence of cobalt in animal tissues, especially the pancreas, was demonstrated conclusively in 1925 (83). This early study gave no definite indication as to the physiological function of cobalt. Four years later, however, researchers found that the administration of cobalt in relatively large but sublethal doses stimulated hematopoiesis and induced polycythemia in rats (84).

In 1948, the pure antipernicious anemia factor was discovered to be a cobalt containing compound (85, 86) soon after designated as vitamin B_{12} and later termed cyanocobalamin. It was isolated from mammalian liver as a red crystalline compound containing 4.0 per cent cobalt.

The interest in cobalt aside from its presence in cyanocobalamin rests on its value as a non-specific erythropoietic stimulant in the treatment of various types of anemia (87, 88).

Although precise determinations of the cobalt content of the blood are extremely difficult to perform, due to the very low concentrations normally present, Heyrovsky (89) has estimated the cobalt in the plasma and the packed red cells of 16 human subjects. He found the plasma concentration to range from 0.28 to 1.70 mcg. per 100 ml. of blood with the packed cells containing from 0.60 to 2.32 mcg. of cobalt per 100 ml. It has been reported (90) that cobalt does not affect the nature of hemoglobin or its oxygen carrying capacity, nor does it produce a cobalt-containing methemoglobin. Other workers, however, have observed the presence of methemoglobin when normal human blood was incubated at 37° for twenty-four hours in the presence of 0.2 per cent cobalt chloride (91).

Copper

The presence of copper in animal tissues has been known since the work of d'Boutigny (92) in 1833. It was not until 1921, however, that the first evidence of a physiological function of the element was indicated through investigation. In that year, Bodansky (93) discovered the presence of copper in the human brain and in 1925, McHargue (94) presented figures of the copper content of blood and tissues, emphasizing the richness of the liver in this element.

In 1928, Hart and co-workers (95) announced that copper in addition to iron is essential in the formation of hemoglobin in rats. This discovery was soon thereafter verified by another worker (96) who further established that copper is essential for hematopoiesis in a wide range of animal species. In 1940, Schultze (97) stated that the evidence indicated that copper facilitates both the mobilization of iron from the tissues and the utilization of iron by the blood forming organs.

A number of copper-protein compounds have been isolated from both animal and plant sources, several of which were shown to be enzymes with oxidase functions. Recently, the enzyme butyryl CoA dehydrogenase was identified as a cuproflavoprotein in which copper occurs as part of the prosthetic group (98).

The copper in the blood is generally equally distributed between the cells and the plasma (99). The copper content of erythrocytes has been shown to be of the order of 0.000075 per cent. The copper content of the leukocytes and platelets is considerably less, about one-fourth of that in the red blood cell (100). In human blood plasma, 90 per cent or more of the copper exists in the form of a blue copper-protein compound termed ceruloplasmin (101). The remaining plasma copper is thought to be loosely bound to a protein since it is mainly nondialyzable (102).

Several investigations with radioactive copper have been made of its absorption, distribution and excretion for various species of animals, all tending to demonstrate the poor absorption of orally administered copper compared to injected copper (102).

Manganese

Bertrand and Medigreceanu (103), in 1913, were among the earliest workers to show that manganese is constantly present in the tissues and organs of man and animal. It has been shown by dialysis of liver and kidney homogenates, that appreciable amounts of manganese are loosely held in these tissues although some of it is more firmly bound (104). The mechanism of attachment is believed to be preferential affinity of manganese for cell wall protein. Recently, manganese has been shown to be an essential component of arginase, an enzyme of the liver, capable of splitting arginine to form urea and ornithine (105).

The subcutaneous introduction of manganese salts into rabbits has led to their accumulation in all organs, mainly the kidneys, liver, brain, and especially the blood (106). It has been shown that manganese is more readily absorbed when administered parenterally than when taken orally (107, 108).

Data on the manganese content of the blood are exceedingly meager, due to the difficulty encountered in the determination of the low concentrations normally present. Kehoe and co-workers (109) stated that whole human blood contains 0.12 to 0.18 mcg. of manganese per ml., about two-thirds of which is present in the corpuscles.

Zinc

The presence of zinc in the muscles and liver of man was first established in 1877 (110). Since then, zinc has been found in all of the tissues and organs of the body that have been examined for it, some investigators believing it to occur in all living cells (111).

Several early attempts were carried out without success to demonstrate an essential function of zinc in mammalian physiology (112, 113, 114). In 1939, direct experimental evidence showed this element to be present in the prosthetic group of the enzyme carbonic anhydrase (115). Carbonic anhydrase catalyzes the reaction, $H_2O + CO_2 \rightleftharpoons H_2CO_3$, therefore playing an important role in the acid-base equilibrium of the body by catalyzing the conversion of carbon dioxide from the tissues to carbonic acid in the blood which is subsequently broken down to release carbon dioxide in the lungs (111).

Vallee and Gibson (116) studied the zinc content of human blood and found 8.8 ± 2.0 mcg. of zinc per ml. of whole blood. Calculations showed that 75 per cent of the total zinc in the blood was situated in the erythrocytes, 22 per cent in the plasma and 3 per cent in the leukocytes. The individual leukocyte, however, contained 25 times as much zinc as the individual erythrocyte. Under normal conditions, the whole of the zinc in erythrocytes can be accounted for as carbonic anhydrase (117).

The concentration of zinc in the peripheral leukocytes of patients with certain types of leukemia has been shown to be greatly reduced below normal levels. Injections of zinc gluconate have failed to raise the zinc level in such cases (118).

Since no carbonic anhydrase activity has yet been shown for the plasma, the zinc present in the plasma is thought to exist in some other manner (119). It seems possible that zinc may occur in combination with one or more plasma proteins, since patients with albuminuria excrete, on the average, seven times as much zinc in their urine daily as do normal individuals (120). It has been demonstrated that after intravenously injecting a zinc salt into a rabbit, the zinc content of the erythrocytes rises only as long as the plasma level remains elevated (121).

Metallic Cations and Blood

Permeability of Erythrocytes

The permeability of the red cell membrane to various electrolytes has been thoroughly reviewed by previous workers (122, 123). In this section, additional information concerning the hemolytic effects and membrane penetration by metallic cations will be presented.

In the earlier literature, the erythrocyte was treated as a cation-impermeable cell (124). With the advent of the isotopic tracer techniques, the ability of certain cations to penetrate the red cell membrane was demonstrated (125, 126, 127).

Potassium and rubidium chlorides were demonstrated to be less effective than sodium chloride in preventing osmotic hemolysis of human blood whereas cesium and lithium chlorides were shown to be more effective (128). Although the red cell membrane is impermeable to sodium under normal conditions, potassium is able to slowly traverse it. The actual exchange of intracellular potassium is equivalent to approximately 1 per cent per hour at body temperature (129).

Ruyssen and Croes (130) found that alkaline earth cations inhibited hemolysis by both digitonin and hypotonic solutions in the following order of increasing activity: magnesium, calcium, strontium, barium. Alkaline earth ions were said to thicken the erythrocyte membrane by forming insoluble salts with the phosphatide acids present in the cell wall.

Croes (131) later found that alkali earth cations increased the rate of hemolysis induced by saponin in the following order: magnesium, calcium > strontium > barium. Although magnesium and calcium increased the rate of hemolysis caused by sodium cetyl sulfate and sodium lauryl sulfate, they had no effect on hemolysis induced by cetyltrimethylammonium bromide.

In the presence of lead (132), erythrocytes lost potassium and became less resistant to hemolysis by hypotonic solutions, the effect increasing with an increase in the concentration of lead. The effect was attributed to an action on the cell membrane. At constant lead concentration, decreased pH indicated potassium loss.

Meneghetti (133) stated that the action of metallic salts on erythrocytes depends on both the concentration and activity of their cations. He showed that extremely small amounts of metals sufficed to produce hemolysis, the hemolytic activity increasing in the following order: zinc < cadmium < iron, cobalt < nickel, platinum < lead < copper, mercury, palladium < gold < silver.

Hess and Reitler (134) noted that sodium chloride solutions which were in contact with metals had hemolytic effects. The degree of hemolysis depended on the metal, the temperature, the concentration of the red blood corpuscles, and on the duration of contact between the metal and the solution. The hemolytic activity seemed to decrease in the following order: copper > lead > silver > nickel > molybdenum > antimony > tin > aluminum. The hemolysis seemed to increase with the dilution of the erythrocyte suspension and was inhibited when serum was substituted for the saline solutions.

It has been shown (135) that iron, copper, lead, and zinc salts all affected hemolysis, with zinc salts demonstrating the highest hemolytic activity. The valence state of the metal seemed to be related to the hemolytic power of the salt, the lower the valence state the greater the hemolytic power.

In a study of the hemolyzing action of copper salts on rabbit erythrocytes, Lambin and co-workers (136) suspended washed red cells in isotonic saline or sucrose solutions. They found that copper sulfate, citrate, tartrate, benzoate, phenylpropionate, and phenylbutyrate all acted similarly; they hemolyzed the erythrocytes in concentrations above 1.3×10^{-5} mole/liter, caused agglutination in concentrations of from 1×10^{-2} to 1×10^{-5} mole/liter and were non-hemolytic in concentrations of 6.6×10^{-6} mole/liter or less. They concluded that the anion was not a factor in the effect of copper salts on erythrocytes treated in such a manner. They later reported that copper salt concentrations lower than 1.3 x 10^{-5} mole/liter had no effect on the resistance of red blood cells to hypotonic solutions (137).

Employing the hemolytic method for the determination of i values, Hartman and Husa (4) suggested that zinc sulfate may make the red blood cell more resistant to hemolysis in hypotonic solutions by strengthening the erythrocyte membrane. Cadwallader and Husa (5) recently noticed unusual behavior in hemolytic experiments involving zinc acetate; low concentrations of the zinc salt seemed to protect the erythrocytes against osmotic hemolysis. Extremely high i values were obtained for both zinc acetate and zinc sulfate by the investigators.

Interactions with Proteins

Metallic cations are found intimately associated with proteins throughout biological systems. Gurd and Wilcox (138) explained that the nature of various metal-protein complexes differs greatly depending on the characteristics of the two individual components. They stated that proteins with their many reactive side chains have the potential capacity to bind most metals very tightly, so that any metallic cation that enters a living organism spends an important part of its time bound to proteins.

In metal-protein complexes, side chain groups as carboxyl, imidazole, and sulfhydryl are usually of much more importance in binding the metal ions than are the terminal amino and carboxyl groups. Changes in the configuration of a specific protein may create new sites, as in the case of serum albumin which shows a large increase in its binding capacity for zinc when it is warmed from 25° to 37° (139).

Metalloproteins are recognized as a structural type in which a specific metal atom takes an integral part in the structure of the protein. The metal in such cases cannot be removed without destroying the protein structure. The nature of metalloproteins has recently been reviewed by Vallee (140).

In some metal protein complexes the metal ion is bound reversibly, in which case physical properties such as solubility and aggregation may be affected. The addition of metal salts whose metal ions interact appreciably with the protein usually lowers the solubility of the protein (141).

There are two general types of metal-protein complexes, simple complexes in which each metal ion combines with a single ligand group and chelate complexes in which each metal ion combines with two or more ligand groups. Each type may affect the solubility of the protein; for example, isoelectric human hemoglobin at pH 6.6 and 0° is much less soluble after the addition of zinc acetate than in a similar medium containing only sodium salts (141). On the other hand, transferrin-$(Fe^{III})_2$, a chelate metal protein complex, is much more soluble than the metal free protein under certain conditions (142).

Changes in the solubility of metal-protein complexes with changes in pH are well known. At 0°, in the presence of 0.01 to 0.05 M. concentrations of lead and zinc salts, it was found that the solubility of serum albumin was greatly decreased over the pH ranges of 2 to 8 for the salts of lead and 5 to 8 for the zinc salts (141). In the same study, the effectiveness of some transitional elements in precipitating human serum albumin in the pH range of 6 to 7 was found to be as follows in decreasing order of effectiveness: copper, cadmium, zinc, and nickel.

In general, the addition of small proportions of metal salts to protein solutions results in the rapid formation of very bulky precipitates (143, 144).

Anson and Mirsky (145) have stated that hemoglobin is a typical coagulable protein, the processes of denaturation resembling those of other proteins. They have theorized that hemoglobin is depolymerized into hemochromogen. These workers stated that the denaturation of hemoglobin is a reversible procedure but that the flocculation of the denatured protein makes it appear to be irreversible. They found hemoglobin to be denatured by heat, acid, alkali, alcohol, mechanical shaking, potassium iodide, potassium thiocyanate, sodium salicylate, and concentrated urea solutions.

Wu and co-workers (146) have mentioned that reversible hemoglobin denaturation, called pseudodenaturation, affects the prosthetic group only therefore being fundamentally different from the denaturation of other proteins. They found that hemoglobin denatured by shaking could not be reverted back to its original form.

Rawlinson (147), in 1938, studied the effect of the divalent ions of copper, zinc, and mercury on the protein of rabbit blood pigment. He determined the range of pH within which globin and oxyhemoglobin were precipitated by small amounts of zinc sulfate, copper sulfate, and mercuric acetate. Globin was completely precipitated by zinc ion at pH 7.5 to 9.6, by cupric ion at pH 7.0 to 8.2, and by mercuric ion at pH 6.3

to 9.0. Oxyhemoglobin was completely precipitated by zinc ion at pH 7.0 to 8.1, by cupric ion at pH 7.0 to 8.3, and by mercuric ion at pH 7.0 to 10.0. The precipitate of the zinc-oxyhemoglobin compound was of a reddish color, remained stable for months, and was readily soluble in either dilute acetic acid or dilute ammonia. The protein appeared to have suffered no permanent change as a result of its precipitation. It showed the normal spectrum for oxyhemoglobin and the oxygen could be removed by repeated equilibriation with pure hydrogen. The mercuric-oxyhemoglobin compound precipitated with the minimal amount of mercuric acetate remained apparently unchanged for some time whereas that precipitated with a large excess of the salt caused the pigment to undergo a change analagous to the formation of parahematin. The copper-oxyhemoglobin was almost immediately changed to a brownish substance regardless of the proportion of the copper salt used to precipitate it. The brownish substance in alkaline solution had a spectrum somewhat similar to that of alkaline hematin and on reduction with sodium hydrosulfite the spectrum was that of hemochromogen.

The Permeability of The Red Cell Membrane

The permeability of the red cell membrane to various organic and inorganic compounds has been reviewed by recent workers (8, 122, 123). The purpose of this section is to briefly consider the permeability of the erythrocyte membrane to organic anions so that a better understanding of the possible activity of the gluconate ion may be reached.

The red cell is recognized as being quite permeable to water and to some anions, but only slowly permeable to cations; the permeability of the membrane to anions is estimated as being about 10^6 times that to potassium (148). Ponder (148) explained that rapid volume changes, induced by changing the concentration of salts or of nonelectrolytes in the suspension medium, tend to be brought about by the movement of water alone, that is, the cell tends to behave as an osmometer.

Hober (149) recognized the incompleteness of our understanding of erythrocyte permeability to organic anions. He presented several factors that must be considered in explaining the osmotic changes of cell volume and hemolysis; these factors are: a) whether the organic acids are surface active, b) whether the organic acids forming the salts are strong or weak, c) whether the organic acids are lipoid soluble, d) whether the ions are strongly hydrated, e) whether the ions have hydrotropic or solubilization properties, and f) whether the ions are poisonous, acting either on the surface or on the entire protoplast.

Cadwallader and Husa (6) recently reported that the order in which some organic anions appeared to protect rabbit erythrocytes against osmotic hemolysis was tartrate, succinate > citrate > p-aminobenzoate > acetate, salicylate, and benzoate. For human blood, the order of protection appeared to be tartrate > succinate > p-aminosalicylate > p-aminobenzoate > salicylate > benzoate, with citrate falling between succinate and p-aminobenzoate, depending on concentration. They noticed that the protective action of the organic anions seemed to increase with an increase of polar groups present; increased hydration, with slower diffusion and increasingly difficult traversing of membrane pores, was offered as a possible explanation. Hydration of the anion could understandably increase the effective concentration of a solute by decreasing the amount of available water in a particular solution. Such a solution would have an increased tonicity.

Effect of pH on Hemolysis

Hemolysis of erythrocytes in hypotonic media must be differentiated from that caused by a change in the equilibrium of the blood constituents with respect to pH. The osmotic fragility of the red blood cells may be increased and their permeability to various ions altered with a change in pH.

Konikov (150) found that the resistance of erythrocytes to the hydrogen ion varied with the animal species. The guinea pig erythrocytes showed the greatest resistance, hemolyzing at pH 3.7, whereas erythrocytes from ruminants hemolyzed at pH 6.0. This variation in resistance was ascribed to the fact that erythrocytes of various species contain stroma composed of proteins with different isoelectric points; the stroma which are less prone to agglutination are more strongly hydrophil and consequently promote hemolysis.

Christophers (151) claimed that the hemolytic effects of acids and bases depend on some critical hemolytic dose absorbed by the cells, at which point acid dissociable compounds form with the cell proteins.

According to Harris (152), when cells are placed in a medium containing either anions which do not penetrate rapidly or a low concentration of electrolyte, there is a change in the intracellular pH with a potential difference developing across the cell membrane. If erythrocytes are introduced into such media there is an exchange of internal chloride ions for external hydroxyl ions making the cell interior alkaline and the external solution acid. The alkalinity, if excessive, can cause hemolysis; for this reason cells keep better in medium acid buffered than in neutral or alkaline solutions. When cells become moderately alkaline, the ionization of their hemoglobin is increased necessitating fewer anions to maintain electrical neutrality within the cell. Also resulting are a decrease in the internal osmotic pressure of the cell, a decrease in the cell volume, and an increase in the resistance of the cell to lysis by hypotonic solutions (153).

Human red blood cells in saline media containing acetate buffers begin tocrenate at pH 7.0, as the pH is reduced towards the isoelectric point of hemoglobin, pH 6.6 to 6.8, and the crenations persist until about pH 6.0, below which the cells are discoid until they begin to hemolyze at pH 5.4 Crenation under these conditions appears to be associated with intracellular crystallization of hemoglobin. Crenation also occurs above pH 8.0 and the cells assume a perfectly smooth spherical form at pH 9.2 (154).

It has been demonstrated (155) that the volume of erythrocytes gradually increases with the addition of sulfuric acid until hemolysis results. In osmotic hemolysis, hemoglobin flows out of the cells whereas in acid induced hemolysis a portion of the hemoglobin remains fixed in the stroma.

Bodansky (156) found the order of effectiveness of inorganic acids in producing hemolysis of human and dog erythrocytes to be: phosphoric, sulfuric, hydrochloric, and nitric acids, listed in order of decreasing effectiveness. He found human red corpuscles to be less resistant than canine red cells to hemolysis caused by acidity. In concentrations causing rapid hemolysis, i.e., pH 1.0 to 2.8, there was injury to the cell membrane, beyond that which could occur in osmotic hemolysis. He showed that in hemolysis induced by inorganic acids, a reaction between the cell constituents and the acid was complete before hemolysis began; this was in part ascribed to the rapid penetration of the acids into the cell.

In 1931, Jacobs and Parpart (157) showed that changes in pH as little as 0.01 of a unit and temperature variations as small as 0.5 of a degree could have a measurable effect on the observed degree of hemolysis. In a later paper, Parpart *et al*. (158) demonstrated that by lowering the pH from 6.79 to 6.59, a salt concentration of 0.525 per cent was needed to protect 50 per cent of the suspended erythrocytes from hemolyzing whereas only 0.500 per cent salt concentration had been needed prior to lowering the pH.

Human erythrocytes have been shown to become permeable to lithium and sodium ions at pH 6.67, to potassium ions at pH 6.8, to rubidium ions at pH 6.92, to cesium ions at pH 7.06, and to low concentrations of ammonium ions at pH 7.48 (159). The passage of chloride and sulfate ions through the cell membrane has been shown to be slowed by lowering the pH of the solution in which the cells are suspended to below pH 7.4 (160).

Davson and Reiner (161) found that the cat red blood cell has a sharp pH optimum (pH 7.2) for sodium permeability; the effects of pH on potassium permeability were small in comparison. The cat erythrocyte normally contains a high concentration of sodium as its main intracellular cation in contrast to the human erythrocyte which contains mainly potassium.

High concentrations of hydrogen ions or hydroxyl ions are capable of producing irreversible changes in the erythrocytes themselves, although over a considerable part of the pH range the effects are reversible.

EXPERIMENTAL

Chemicals

The chemicals used in this investigation were obtained from commercial sources. The chemicals are listed in Table 1 along with their sources, formulas, grades, and lot numbers. The abbreviations used and their meanings are as follows:

A	Amend Drug and Chemical Co., Inc.
C	Chemo Puro Manufacturing Corp.
M	Mallinckrodt Chemical Works
MK	Merck and Co., Inc.
P	Chas. Pfizer and Co., Inc.

Methods

Collection of Blood

The rabbit blood used was obtained from healthy male and female rabbits by heart puncture. A 17-gauge, 2-inch sterile disposable needle attached to a 10-ml. sterile syringe was employed to make entrance into the heart. The cardiac puncture was achieved through the left intercostal spaces as near to the sternum as possible in order to avoid injury to the pleural cavity. Approximately 10 ml. of blood was withdrawn and immediately defibrinated by gentle rotation with 10 to 15 glass beads contained in a 50-ml. Florence flask. After swirling the

TABLE 1

CHEMICALS USED IN PREPARING SOLUTIONS

Chemical	Source	Formula	Grade	Lot No.
Calcium Gluconate	A	$Ca(C_6H_{11}O_7)_2$	USP XV	G51716
Cobalt Gluconate	C	$Co(C_6H_{11}O_7)_2\cdot 4H_2O$	Refined	8320
Copper Gluconate	P	$Cu(C_6H_{11}O_7)_2$	Refined	5998
Iron Gluconate	A	$Fe(C_6H_{11}O_7)_2\cdot 2H_2O$	NF IX	E53797
Magnesium Gluconate	P	$Mg(C_6H_{11}O_7)_2\cdot 2H_2O$	Refined	5208
Manganese Gluconate	P	$Mn(C_6H_{11}O_7)_2\cdot 3H_2O$	Refined	5448
Potassium Gluconate	P	$KC_6H_{11}O_7$	Refined	51368
Sodium Carbonate	M	Na_2CO_3	Reagent	CJR
Sodium Chloride[a]	MK	NaCl	Reagent	51587
Sodium Gluconate	P	$NaC_6H_{11}O_7$	Refined	G51118
Zinc Gluconate	C	$Zn(C_6H_{11}O_7)_2\cdot H_2O$	Refined	7704

a - Sodium chloride for biological work.

flask for approximately five minutes, the defibrinated blood was decanted into a 50-ml. Erlenmeyer flask and aerated by gentle rotation of the flask for about five minutes.

The human blood was obtained chiefly from the veins of the arms of a twenty-five year old white male. Several experiments were conducted employing blood donated by male volunteers. The blood was drawn from the arm of the donor with a hypodermic syringe and needle and treated in the same manner as the rabbit blood.

Fresh blood samples were used throughout this investigation.

Preparation of Solutions

Solutions of the chemical substances were prepared by weighing the proper amounts of the chemicals and placing them in volumetric flasks which were brought to volume with triple distilled water. All of the chemical concentrations were calculated in terms of the anhydrous salt.

An approximate dilution range was determined for each salt by conducting a preliminary experiment in which the concentration of chemical sufficient to prevent hemolysis and the maximum concentration present without preventing hemolysis constituted the limits of the desired range. Various intermediate concentrations were then prepared and the experiment carried out.

In experiments employing solutions containing both sodium chloride and the chemical under investigation, the same procedure was followed.

Quantitative Determination of Per Cent Hemolysis

The method used to determine the degree of hemolysis was essentially that of Hunter (162). It is based on the fact that the quantity of red oxyhemoglobin exuded from the erythrocytes by a hypotonic solution is a direct function of the proportion of red corpuscles hemolyzed. The amount of oxyhemoglobin liberated on complete hemolysis, by the laking of erythrocytes in 0.1 per cent sodium carbonate solution, represents unity in expressing the degree of hemolysis occurring in a given strength of salt solution. The quantitative determination of the proportion of hemolysis in a salt solution of any strength is achieved by centrifuging down any unhemolyzed cells just prior to determining the amount of oxyhemoglobin present in the supernatant solution by means of a photoelectric colorimeter.

The method employed in these experiments consisted of volumetrically transferring duplicate 5 ml. portions of standard sodium chloride solutions, ranging in concentration from 0.32 to 0.44 per cent for Caucasian blood, 0.24 to 0.44 per cent for Negro blood and 0.32 to 0.52 per cent for rabbit blood into colorimeter tubes. To these solutions, 0.05 ml. of fresh defibrinated, aerated blood was added and the tubes carefully inverted several times to obtain complete mixing. The blood-salt mixtures were then placed in a water bath at 37° for forty-five minutes. At the end of this period the mixtures were centrifuged for three minutes at approximately 2000 r.p.m. A Klett-Summerson photoelectric colorimeter with a No. 54 green filter was used to measure the amount of light absorbed by the supernatant liquid.

Complete hemolysis of human and rabbit blood was effected by placing 5 ml. of a 0.1 per cent sodium carbonate solution in a colorimeter tube, adding to it 0.05 ml. of blood and mixing. After allowing the tube to stand for the prescribed forty-five minutes at 37°, the preparation was centrifuged for three minutes and the amount of light absorbed determined colorimetrically. Five such tubes were prepared for each blood sample, two being read at the beginning and three at the end of the experimental run. The average of the five readings represented the 100 per cent hemolysis value and was subsequently employed in calculating the degree of hemolysis of the blood in the various salt concentrations.

A blank was prepared by placing 0.05 ml. of blood in 5 ml. of 0.85 per cent sodium chloride solution, allowing to stand for forty-five minutes and centrifuging. The colorimeter was calibrated to zero by utilizing two such prepared blanks.

The per cent hemolysis occurring in duplicate tubes of each strength of salt solution was calculated by dividing the average readings obtained from the two readings of each salt concentration by the average reading obtained from the complete hemolysis obtained with sodium carbonate solution. These calculations multiplied by 100 then represented the per cent of total hemolysis in each strength of salt solution.

Variations in erythrocyte osmotic fragility in solutions of the same salt concentration with different samples of human blood has been noted (2). Any error due to this variation was minimized by determining the degree of hemolysis for each blood sample in sodium chloride solutions of varying concentration. Thus a direct comparison between the degree of hemolysis occurring in a certain concentration of sodium chloride solution and a solution of some other salt was obtained.

Determination of Per Cent Hemolysis When Dealing with Colored Solutions

The solutions of several gluconates studied in this work were colored. For the quantitative determination of the per cent hemolysis when dealing with such solutions, one of two methods was employed depending on whether or not the color of the solution produced an additive colorimetric reading with oxyhemoglobin.

In the first method oxyhemoglobin solution prepared by laking erythrocytes in triple distilled water was added in like amounts to two colorimeter tubes, one containing 5 ml. of triple distilled water and the other containing 5 ml. of the colored gluconate solution. To a third tube, containing 5 ml. of the colored solution, a portion of triple distilled water equal in volume to the oxyhemoglobin solution placed in each of the first two tubes was added. The colorimeter reading of this third tube added to the reading of the tube containing only water and oxyhemoglobin was compared to the reading of the tube containing the oxyhemoglobin and the colored gluconate solution; if the readings agreed within experimental error the two colors were considered to be additive. If this were found to be the case, colorimeter readings of each gluconate solution were made before the addition of blood and served as blanks. Their values were subtracted from the colorimetric readings obtained after the blood was added to permit an accurate determination of the amount of light being absorbed only by the oxyhemoglobin present in the supernatant liquid.

If the colors of oxyhemoglobin and the gluconate solution were not found to be additive, the second of the two methods devised was employed. In this method the per cent hemolysis was determined by difference, that is, from the amount of oxyhemoglobin remaining in the erythrocytes not yet hemolyzed. The standard procedure employed when dealing with colorless solution was carried out until the point following centrifugation of the tubes, just prior to the time that the colorimetric readings were to be taken. Instead of taking the readings, the supernatant solution of the colored gluconate and oxyhemoglobin from laked cells was meticulously decanted from each tube, being careful not to disturb the unhemolyzed cells clinging tenaciously to the bottom. The unhemolyzed cells were then washed with 0.85 per cent sodium chloride solution, recentrifuged and decanted, the process being repeated until the supernatant liquid was free of color. The supernatant liquid of the last washing was decanted and

0.1 per cent sodium carbonate solution added to a mark etched on the colorimeter tube, denoting a volume of 5.05 ml. The tube was gently inverted to insure complete hemolysis, the ghost cells centrifuged down, and the resulting color read on the colorimeter. By subtracting the average reading of two duplicate tubes from the reading of 100 per cent hemolysis, the colorimetric value of the oxyhemoglobin exuded from the cells originally hemolyzed was obtained. From this, the per cent hemolysis was calculated in the usual manner.

Calculation of i Values for Use in Preparation of Isotonic Solutions

In order to determine the i value of the gluconates used in this study, the per cent hemolysis of a blood sample in varying concentrations of sodium chloride solution as well as in varying concentrations of gluconate solution was determined. By plotting the per cent hemolysis against the strength of the solution, a sigmoid type of curve invariably resulted with sodium chloride solutions, and usually for solutions of other salts. From the curves showing the degree of hemolysis in both the sodium chloride and the gluconate solutions, the concentrations in Gm./100 ml. of sodium chloride and the gluconate (causing 25, 50, and 75 per cent hemolysis) were obtained.

Knowing the concentration of sodium chloride and any other compound giving the same degree of hemolysis, the hemolytic i value for the compound being investigated may be calculated according to the following equation:

$$\frac{\left(\begin{array}{c}\underline{i}\text{ value for}\\ \text{NaCl}\end{array}\right)\left(\begin{array}{l}\text{Gm. of NaCl}\\ \text{in 100 ml.}\\ \text{of solution}\end{array}\right)}{(\text{Gm.-molecular weight of NaCl})} = \frac{\left(\begin{array}{l}\underline{i}\text{ value for}\\ \text{other com-}\\ \text{pound}\end{array}\right)\left(\begin{array}{l}\text{Gm. of other}\\ \text{compound in 100}\\ \text{ml. of solution}\end{array}\right)}{(\text{Gm.-molecular weight of compound})}$$

In using the above equation the value of *i* for sodium chloride was taken as 1.86, the accepted value for 0.9 per cent (0.15 M) sodium chloride solution. This *i* value of sodium chloride was calculated from activity coefficients. The *i* values of the gluconates used in these experiments were calculated from concentrations causing 25, 50, and 75 per cent hemolysis of human and rabbit erythrocytes.

In calculating *i* values for gluconates determined in the presence of 0.2 per cent sodium chloride, 0.2 Gm. per 100 ml. of sodium chloride was subtracted from the concentrations of sodium chloride causing 25, 50, and 75 per cent hemolysis. The average of these three *i* values was considered to be a representative value. A minimum of two such average values was obtained for both human and rabbit blood for each compound determined.

pH Experiments

In preliminary hemolysis experiments employing some iron salts, a darkening of the blood was observed on several occasions. It was suggested that one possible cause of such darkening was the acidity of the solutions being tested (163, 164). Since the entire hemolytic method depends on the accurate colorimetric determination of cherry-red oxyhemoglobin, any color change would make such determinations worthless. An experiment was carried out to determine the maximum acidity that a solution may have without altering the color of the oxyhemoglobin liberated from hemolyzed erythrocytes.

By volumetrically adding equal quantities of defibrinated rabbit blood to one test tube containing triple distilled water and to another tube containing an equal volume of acidified triple distilled water, colorimetric comparisons of the liberated oxyhemoglobin was possible. By varying the degree of acidity of the water in a series of tubes, the pH at which the blood failed to produce the same colorimetric reading as the tube containing unaltered triple distilled water was easily determined.

In another experiment, the effect of increasing the hydrogen ion concentration on hemolysis was determined. The pH range covered was that which would include the pH values of those gluconate solutions which were more acidic than the reference sodium chloride solutions employed; this range was pH 3.5 to 6.2. The method employed consisted of volumetrically transferring duplicate 5 ml. portions of standard sodium chloride solutions, ranging in concentration from 0.32 to 0.52 per cent, into colorimeter tubes. The pH values of these solutions were determined with a battery operated Beckman pH meter. Like portions of sodium chloride solutions of the same concentrations but acidified with concentrated hydrochloric acid to various degrees of acidity were volumetrically transferred to colorimeter tubes. To each of the tubes 0.05 ml. of fresh defibrinated rabbit blood was added and the tubes carefully inverted to obtain complete mixing. The tubes were then placed in a water bath at 37° for forty-five minutes after which they were treated in the usual manner for the determination of per cent hemolysis of the blood in each tube. By plotting the per cent hemolysis against the concentration of the solutions, sigmoid curves resulted for both the data obtained from the acidified as well as the unacidified sodium chloride solutions. A direct comparison of the shapes of the curves and their relative positions provided a means of attributing a portion of the hemolysis occurring in the blood-gluconate mixtures to the hydrogen ion concentration.

Determination of Metal-Protein Precipitation

Before an attempt was made to determine hemolytic *i* values, a preliminary test was run to determine whether or not precipitation occurred when a solution of oxyhemoglobin was added to a solution of the gluconate. One per cent solutions of each gluconate were separately mixed with equal portions of oxyhemoglobin solution prepared by laking washed erythrocytes, allowed to stand in a water bath for forty-five minutes at 37°, centrifuged, and observed for evidence of precipitation. During the course of a hemolytic run, such precipitation would cause the colorimetric readings to be proportionally lower than those which would result from the proportion of oxyhemoglobin actually released from the laked erythrocytes.

Additional experiments were carried out to determine whether or not serum proteins were precipitated by the gluconate solutions. Serum was separated from blood cells by centrifugation and added in like portions to 1 per cent solutions of each gluconate. The mixtures were observed repeatedly for forty-five minutes during which time they were in a water bath at 37°.

During each determination of hemolytic *i* values, the gluconate-blood mixtures were observed for evidence of precipitation.

Freezing Point Determinations

The apparatus used to determine the freezing points of solutions of various salts of gluconic acid was that of Bartley (165) as modified by Husa and Adams (1). The modification of the original apparatus was merely the elimination of the toy motor employed to run the stirring wire.

The apparatus, in part, consisted of a vacuum Dewar tube, 22 cm. high and 6 cm. inside diameter, held upright by a ring and ringstand. The tube was fitted with a rubber stopper having a large hole in the center and three smaller holes around the edge. A heavy glass test tube, about 20 cm. long and 3 cm. inside diameter was fitted into the large hole and passed down into the Dewar tube. Into two of the smaller holes were fitted copper tubes, one terminating just below the under surface of the stopper. The other copper tube ran to the bottom of the Dewar tube ending in a coil, the coil being perforated with a series of small holes. Inside the test tube was a second glass tube of slightly smaller dimensions and supported by a ring of rubber tubing placed between the lips of the two tubes. There was a very small space between the surfaces of the two tubes. An ordinary laboratory thermometer was inserted into the fourth hole in the stopper and was used to observe the temperature of the outer bath during freezing point determinations. A Beckmann differential thermometer with a platinum wire stirrer looped loosely around its bulb was placed in the innermost tube and held by means of a rubber stopper.

Prior to operating the apparatus, the coiled copper tube was connected to an air line by means of rubber tubing. The short copper tube was fitted with a section of rubber tubing which terminated out of doors, thus serving as the exit for vaporized ether. About 50 ml. of the solution to be tested was placed in the innermost tube; this quantity was sufficient to cover the bulb of the Beckmann thermometer. A quantity of ethyl alcohol was added to the middle tube until its thin layer rose to a level just above that of the solution in the inner tube. In the Dewar tube was a liberal supply of ether, its surface approximately level with the other liquids. During the determinations, air was passed through the ether causing it to evaporate at a rate controlled by the intensity of aeration, the vaporization of ether being the basis for the cooling process.

The temperature of the ether bath was lowered to approximately -3° before there was evidence of movement of the mercury column in the Beckmann thermometer. In order to freeze the solvent portion of the test solution, it was necessary to reduce the temperature of the ether bath to about -10°. By carefully controlling the rate of aeration, the cooling process was performed at a slow steady pace. During the cooling operation, each sample was stirred vigorously and continuously but not so as to strike the thermometer bulb or to carry warm air down into the liquid. When the solvent began to freeze and separate from the solution, the stirring rate was increased and maintained until a stationary temperature was reached. Supercooling occurred in each case; i.e., the temperature fell below the freezing point of the solution then rose suddenly to a fixed point and remained there for several seconds before falling once again. The temperature of this equilibrium point was recorded as the freezing point of the solution.

Five hundred milliliters of triple distilled water were used in the preparation of each of the 0.025 and 0.010 molal solutions to be tested. The freezing points of ten 50 ml. samples of each solution were determined and recorded. The point on the scale of the Beckmann differential thermometer which represented the freezing point of water was obtained from the average of 25 separate determinations. Each temperature was accurately read to 0.01 of a degree and estimated to 0.001 of a degree. The Beckmann thermometer was fitted with a magnifying lens to aid the operator. The freezing point depressions of the various solutions were then determined by merely subtracting the freezing point temperature of water from the freezing point temperature of each solution tested.

The freezing point data obtained were employed in the calculations of i values according to the formula

$$\Delta T_f = K_f \underline{i} M$$

where ΔT_f is the change in the freezing point of water after the addition of solute, K_f is the molal freezing point constant for water (1.86), i is the van't Hoff factor, and M is the molality of the solution.

Data of the various hemolytic and freezing point determinations are presented in the following tables.

Data

TABLE 2

READINGS ON KLETT-SUMMERSON COLORIMETER FOR 100 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN 0.1 PER CENT SODIUM CARBONATE SOLUTION

	Colorimeter Readings of Blood Sample									
	1	2	3	4	5	6	7	8	9	10
	350	361	399	355	292	337	404	373	362	321
	346	364	404	373	291	332	405	371	364	321
	352	368	405	371	302	325	399	355	368	318
	354	365	399	361	282	331	399	361	356	324
	348	360	414	361	294	322	414	361	363	321
Average	350	364	404	364	292	329	404	364	363	321

(Table continued on following page)

TABLE 2 (continued)

	Colorimeter Readings of Blood Sample									
	11	12	13	14	15	16	17	18	19	20
	288	300	304	228	306	387	254	344	362	349
	270	297	311	227	296	389	254	339	362	341
	262	300	299	235	288	378	258	339	367	344
	264	303	300	220	308	382	249	341	369	339
	274	300	318	210	304	397	255	349	370	339
Average	272	300	306	224	300	387	254	342	366	342

(Table continued on following page)

TABLE 2 (continued)

	Colorimeter Readings of Blood Sample								
	21	22	23	24	25	26	27	28	29
	367	356	350	339	335	316	346	388	389
	362	360	361	339	334	316	351	386	394
	362	370	340	321	324	309	346	390	398
	369	371	353	329	323	304	344	387	390
	370	374	343	334	329	301	343	389	391
Average	366	366	349	332	329	309	346	388	392

TABLE 3

READINGS ON KLETT-SUMMERSON COLORIMETER FOR 100 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN 0.1 PER CENT SODIUM CARBONATE SOLUTION

	Colorimeter Readings of Blood Sample									
	30	31	32	33	34	35	36	37	38	39
	492	500	500	424	458	479	493	420	399	447
	485	504	508	423	454	478	484	426	404	456
	491	508	494	425	462	469	494	425	409	456
	490	495	484	426	460	471	508	423	400	448
	497	497	493	420	456	482	500	424	403	432
Average	491	501	496	424	458	476	496	424	403	447

(Table continued on following page)

TABLE 3 (continued)

	Colorimeter Readings of Blood Sample									
	40	41	42	43	44	45	46	47	48	49
	500	481	500	467	453	449	454	465	475	458
	492	486	501	467	456	450	455	458	476	465
	492	483	493	469	455	461	459	458	479	458
	490	476	490	476	453	452	460	464	489	464
	496	479	496	466	448	461	456	466	488	466
Average	494	481	496	469	453	455	457	462	481	462

(Table continued on following page)

TABLE 3 (continued)

	Colorimeter Readings of Blood Sample									
	50	51	52	53	54	55	56	57	58	59
	488	341	416	438	489	445	522	468	389	432
	489	337	421	428	487	453	526	478	371	434
	479	346	419	430	485	457	518	473	394	432
	476	344	415	418	492	459	515	480	410	431
	475	342	414	426	482	447	524	464	389	431
Average	481	342	417	428	487	452	521	473	392	432

(Table continued on following page)

TABLE 3 (continued)

	Colorimeter Readings of Blood Sample									
	60	61	62	63	64	65	66	67	68	69
	398	448	456	424	411	532	361	432	456	270
	399	458	444	421	410	528	366	434	458	272
	401	459	431	439	408	521	355	432	458	270
	399	460	441	414	419	529	369	431	450	277
	398	465	446	408	406	535	369	431	458	271
Average	399	458	444	421	411	529	364	432	456	272

TABLE 4

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN SODIUM CHLORIDE SOLUTIONS OF VARIOUS CONCENTRATIONS

Per Cent NaCl	Average Colorimeter Readings[a] of Blood Sample						Per Cent Hemolysis of Blood Sample					
	1	2	3	4	5	6	1	2	3	4	5	6
0.32	352	363	...	...	292	328	100.6	99.9	....	....	100.0	99.7
0.36	348	341	404	366	290	320	99.3	93.7	100.0	100.6	99.3	97.3
0.37	...	...	391	357	...	...	....	....	96.8	98.1	....	....
0.38	334	318	384	354	173	283	95.4	87.4	95.1	97.3	59.3	86.0
0.39	272	307	...	...	141	215	77.7	84.5	....	....	48.3	65.4
0.40	216	290	362	349	125	155	61.7	79.7	89.6	95.9	42.8	47.1
0.41	128	234	...	...	103	99	36.6	64.3	....	....	35.3	30.1
0.42	79	156	289	348	80	50	22.6	42.9	71.5	95.6	27.4	15.2
0.43	35	80	232	347	66	24	10.0	22.0	57.4	95.3	22.6	7.3
0.44	19	43	188	342	56	11	5.4	11.8	46.5	94.0	19.2	3.3
0.46	...	...	102	271	...	...	....	....	25.3	74.5	....	....
0.52	0	0	0	2	0	0	0.0	0.0	0.0	0.6	0.0	0.0

(Table continued on following page)

TABLE 4 (continued)

Per Cent NaCl	Average Colorimeter Readings[a] of Blood Sample						Per Cent Hemolysis of Blood Sample					
	7	8	9	10	11	12	7	8	9	10	11	12
0.32	...	...	369	325	...	...	....	....	101.6	101.3	....	....
0.36	404	366	360	316	267	300	100.0	100.6	99.2	98.4	98.2	100.0
0.37	391	357	...	...	...	...	96.8	98.1	....	....	....	....
0.38	384	354	254	262	227	296	95.1	97.3	70.0	81.6	83.5	98.7
0.39	...	...	223	222	212	292	....	....	61.4	69.2	77.9	97.3
0.40	362	349	173	174	187	274	89.6	95.9	47.7	54.2	68.8	91.4
0.41	...	...	119	123	156	250	....	....	32.8	38.3	57.4	83.3
0.42	289	348	86	74	114	218	71.5	95.6	23.7	23.1	41.9	72.7
0.43	232	347	50	38	68	...	57.4	95.3	13.8	11.8	25.0	....
0.44	188	342	19	16	44	128	46.5	94.0	5.2	5.0	16.2	42.7
0.46	102	271	...	...	15	34	25.3	74.5	....	....	5.5	11.3
0.48	31	112	...	...	4	6	7.7	30.8	....	....	1.5	2.0
0.52	0	2	0	0	...	...	0.0	0.6	0.0	0.0	....	.....

(Table continued on following page)

TABLE 4 (continued)

Per Cent NaCl	Average Colorimeter Readings[a] of Blood Sample						Per Cent Hemolysis of Blood Sample					
	13	14	15	16	17	18	13	14	15	16	17	18
0.32	299	225	299	388	257	349	97.7	100.5	99.7	100.2	101.2	102.1
0.35	283	223	...	...	243	...	92.5	99.6	....	....	95.7	....
0.36	...	...	300	338	...	332	....	.....	100.0	87.3	....	97.1
0.38	260	154	294	295	232	330	85.0	68.8	98.0	76.2	91.3	96.5
0.39	237	128	290	252	205	304	77.5	57.1	96.7	65.1	80.7	88.9
0.40	210	86	280	187	155	275	68.6	38.4	93.4	48.3	61.0	80.4
0.41	190	56	263	154	126	222	62.1	25.0	87.7	39.8	49.6	64.9
0.42	164	34	250	111	81	160	53.6	15.2	83.3	28.7	31.9	46.8
0.43	138	19	202	73	60	109	45.1	8.5	67.4	18.9	23.6	31.9
0.44	116	9	178	42	27	66	37.9	4.0	59.3	10.9	10.6	19.3
0.46	...	...	117	12	9	17	....	....	39.0	3.1	3.5	5.0
0.52	46	0	0	0	0	0	15.0	0.0	0.0	0.0	0.0	0.0

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TABLE 4 (continued)

Per Cent NaCl	Average Colorimeter Readings[a] of Blood Sample						Per Cent Hemolysis of Blood Sample					
	19	20	21	22	23	24	19	20	21	22	23	24
0.32	367	349	367	370	348	327	100.3	102.1	100.3	101.0	99.9	98.5
0.36	321	332	321	...	312	322	87.7	97.1	87.7	....	89.4	97.0
0.38	202	330	202	370	252	313	55.2	96.5	55.2	101.0	72.2	94.4
0.39	151	304	151	369	220	295	41.3	88.9	41.3	100.8	63.0	88.9
0.40	103	275	103	365	163	277	28.1	80.4	28.1	99.8	46.5	83.5
0.41	74	222	74	361	139	239	20.2	64.9	20.2	98.7	39.8	72.0
0.42	32	160	32	...	95	205	8.7	46.8	8.7	....	27.0	61.8
0.43	23	109	23	353	61	132	6.3	31.9	6.3	96.5	17.5	39.8
0.44	12	66	12	350	51	103	3.3	19.3	3.3	95.7	15.2	31.0
0.46	5	17	5	276	17	47	1.4	5.0	1.4	76.0	4.9	14.2
0.48	2	3	2	162	2	5	0.6	0.9	0.6	44.3	0.5	1.5
0.52	1	0	1	3	0	0	0.3	0.0	0.3	0.8	0.0	0.0

(Table continued on following page)

TABLE 4 (continued)

Per Cent NaCl	Average Colorimeter Readings[a] of Blood Sample					Per Cent Hemolysis of Blood Sample				
	25	26	27	28	29	25	26	27	28	29
0.32	331	304	345	386	383	100.1	98.4	99.7	99.5	97.7
0.35	...	264	340	...	...	....	85.4	98.3	....	....
0.36	318	...	...	386	383	96.7	....	....	99.5	97.7
0.38	295	141	298	386	367	89.7	45.6	86.1	99.5	93.6
0.39	285	74	281	366	361	86.6	24.0	81.2	94.3	92.1
0.40	250	44	236	361	355	76.1	14.2	68.2	93.0	90.6
0.41	232	27	198	350	338	70.5	8.7	57.2	90.2	86.2
0.42	202	11	161	310	307	61.4	3.6	46.5	79.9	78.3
0.43	166	5	122	262	...	50.5	1.6	35.3	67.5	....
0.44	117	0	67	230	172	35.6	0.0	19.4	59.3	43.9
0.46	52	...	...	126	67	15.8	....	....	32.5	18.5
0.48	11	...	...	67	24	3.3	....	....	17.3	6.1
0.52	1	0	11	10	5	0.3	0.0	3.2	2.6	1.3

a - Each reading shown is the average of two duplicate tubes.

TABLE 5

PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN SODIUM CHLORIDE SOLUTIONS OF VARIOUS CONCENTRATIONS

Per Cent NaCl	Average Colorimeter Readings[a] of Blood Sample						Per Cent Hemolysis of Blood Sample					
	30	31	32	33	34	35	30	31	32	33	34	35
0.32	475	468	...	...	460	475	96.7	93.4	....	....	100.4	99.8
0.34	...	...	482	397	456	462	....	....	97.2	93.6	99.6	97.0
0.36	474	463	439	371	456	462	96.5	92.4	88.5	87.5	93.9	95.0
0.37	383	287	409	278	...	317	78.0	57.3	82.5	65.6	....	66.6
0.38	274	194	371	241	310	257	55.8	38.7	74.8	56.8	67.7	54.0
0.39	196	124	343	139	177	181	40.0	24.8	69.2	32.8	38.6	38.0
0.40	135	76	280	89	105	112	27.5	15.2	56.5	21.0	22.9	23.6
0.41	83	39	144	46	57	56	16.9	7.8	29.0	10.9	12.4	11.8
0.42	29	23	91	26	16	30	5.9	4.6	18.4	6.1	3.5	6.3
0.44	0	0	20	0	0	4	0.0	0.0	4.0	0.0	0.0	0.8

(Table continued on following page)

TABLE 5 (continued)

Per Cent NaCl	Average Colorimeter Readings[a] of Blood Sample						Per Cent Hemolysis of Blood Sample					
	36	37	38	39	40	41	36	37	38	39	40	41
0.32	...	...	400	418	482	...	....	....	99.3	93.3	97.6	....
0.34	482	397	391	402	...	447	97.2	93.6	97.0	89.9	....	92.9
0.35	...	...	...	...	456	...	....	....	....	....	92.3	....
0.36	439	371	382	337	...	446	88.5	87.5	94.8	75.5	....	92.7
0.37	409	278	296	298	412	359	82.5	65.6	73.4	66.7	83.4	74.6
0.38	371	241	210	224	327	316	74.8	56.8	52.1	50.1	66.2	65.7
0.39	343	139	147	139	210	240	69.2	32.8	36.5	31.1	44.3	49.9
0.40	280	89	79	52	159	140	56.5	21.0	19.4	11.6	32.2	29.1
0.41	144	46	39	24	113	75	29.0	10.9	9.7	5.4	22.9	15.6
0.42	91	26	6	10	47	26	18.4	6.1	1.5	2.2	9.5	5.4
0.44	20	0	0	0	14	0	4.0	0.0	0.0	0.0	2.8	0.0

(Table continued on following page)

TABLE 5 (continued)

Per Cent NaCl	Average Colorimeter Readings[a] of Blood Sample						Per Cent Hemolysis of Blood Sample					
	42	43	44	45	46	47	42	43	44	45	46	47
0.32	...	458	426	446	455	458	....	97.7	94.0	98.0	99.6	99.1
0.34	468	...	...	439	435	449	94.4	....	....	96.5	95.2	97.2
0.36	467	440	360	384	419	373	94.1	93.8	79.5	84.4	91.7	80.7
0.37	452	424	310	323	379	293	91.1	90.4	68.4	71.0	82.9	63.4
0.38	430	390	182	178	315	134	86.7	83.2	40.2	39.1	68.9	29.0
0.39	427	273	91	112	210	73	86.1	58.2	20.0	24.6	46.0	15.8
0.40	417	184	58	74	155	55	84.1	39.2	12.8	16.3	33.9	11.9
0.41	353	106	20	23	83	30	71.2	22.5	4.4	5.1	18.2	6.5
0.42	290	52	12	12	48	16	58.5	11.1	2.7	2.6	10.5	3.5
0.44	169	6	0	2	12	2	34.0	1.3	0.0	0.4	2.6	0.4
0.46	50	...	...	...	...	...	10.1	....	....	....	....	....

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TABLE 5 (continued)

Per Cent NaCl	Average Colorimeter Readings[a] of Blood Sample						Per Cent Hemolysis of Blood Sample					
	48	49	50	51	52	53	48	49	50	51	52	53
0.24	...	...	...	...	410	...	....	....	....	....	98.4	....
0.28	...	...	...	268	378	...	....	....	....	78.4	90.7	....
0.30	...	...	...	...	330	...	....	....	....	....	79.1	....
0.32	458	458	458	200	239	354	95.2	99.1	95.2	58.5	57.3	82.8
0.34	423	449	423	132	66	211	87.9	97.2	87.9	38.6	15.8	49.4
0.36	324	373	324	72	5	67	67.2	80.7	67.2	21.0	1.2	15.6
0.37	245	293	245	48	0	27	50.9	63.4	50.9	14.0	0.0	6.3
0.38	119	134	119	32	0	23	24.7	29.0	24.7	9.4	0.0	5.4
0.39	51	73	51	22	0	10	10.6	15.8	10.6	6.4	0.0	2.3
0.40	32	55	32	18	0	5	6.7	11.9	6.7	5.3	0.0	1.2
0.42	7	16	7	2	0	1	1.5	3.5	1.5	0.6	0.0	0.2
0.44	2	2	2	0	0	0	0.4	0.4	0.4	0.0	0.0	0.0

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TABLE 5 (continued)

Per Cent NaCl	Average Colorimeter Readings[a] of Blood Sample						Per Cent Hemolysis of Blood Sample					
	54	55	56	57	58	59	54	55	56	57	58	59
0.32	465	449	510	472	489	426	95.5	99.3	97.9	99.8	99.9	98.6
0.34	461	...	...	460	...	404	94.7	....	....	97.3	....	93.5
0.36	403	424	482	450	470	403	82.8	93.8	92.5	95.1	95.9	93.3
0.37	262	...	...	384	321	402	53.8	....	....	81.2	65.5	93.1
0.38	159	410	439	254	243	339	32.7	90.7	84.3	53.7	49.6	78.5
0.39	83	370	295	143	164	204	17.0	81.9	56.6	30.2	33.5	47.2
0.40	49	359	190	58	98	44	10.1	79.4	36.5	12.3	20.0	10.2
0.41	30	275	84	33	53	12	6.2	60.8	16.1	7.0	10.8	2.8
0.42	14	139	31	27	32	0	2.9	30.8	5.6	5.7	6.5	0.0
0.44	0	56	3	0	16	0	0.0	14.4	0.6	0.0	3.3	0.0

(Table continued on following page)

TABLE 5 (continued)

Per Cent NaCl	Average Colorimeter Readings[a] of Blood Sample						Per Cent Hemolysis of Blood Sample					
	60	61	62	63	64	65	60	61	62	63	64	65
0.24	383	458	438	414	407	526	96.0	100.0	98.7	98.3	99.0	99.4
0.26	370	448	416	393	394	510	92.7	97.8	93.7	93.4	95.9	96.4
0.28	342	415	408	394	388	501	85.7	90.6	91.9	93.6	94.4	94.7
0.30	302	395	400	377	388	500	75.7	86.3	90.1	89.6	94.4	94.5
0.32	263	360	351	324	365	501	65.9	78.6	79.1	77.0	88.8	94.7
0.34	184	248	216	245	318	456	46.1	54.2	48.7	58.2	77.4	86.2
0.36	42	75	56	142	137	385	10.5	16.4	12.6	33.7	33.3	72.8
0.38	15	7	14	64	53	160	3.8	1.6	3.2	15.2	12.9	30.2
0.40	2	0	0	26	16	55	0.5	0.0	0.0	6.2	3.9	10.4
0.42	0	0	0	6	2	21	0.0	0.0	0.0	1.4	0.5	4.0

(Table continued on following page)

TABLE 5 (continued)

Per Cent NaCl	Average Colorimeter Readings[a] of Blood Sample				Per Cent Hemolysis of Blood Sample			
	66	67	68	69	66	67	68	69
0.24	355	426	...	...	97.5	98.6	....	....
0.26	340	404	...	...	93.4	93.5	....	....
0.28	323	403	...	...	88.7	93.3	....	....
0.30	310	402	...	...	85.2	93.1	....	....
0.32	202	395	433	268	55.5	91.4	95.0	98.5
0.34	61	339	407	274	16.8	78.5	89.2	100.5
0.36	45	204	375	237	12.4	47.2	82.5	87.2
0.38	6	44	161	168	1.6	10.2	35.3	61.8
0.40	0	12	102	85	0.0	2.8	22.4	23.2
0.42	0	0	33	44	0.0	0.0	7.2	11.9
0.44	0	0	13	21	0.0	0.0	2.8	5.7

a - Each reading shown is the average of two duplicate tubes.

TABLE 6

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN SODIUM GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

Per Cent Sodium Gluconate		Colorimeter Readings		Per Cent Hemolysis	
		Blood Sample 1	Blood Sample 2	Blood Sample 1	Blood Sample 2
0.80		351	362		
		347	366		
	Av.	349	364	99.7	100.0
0.90		333	364		
		343	364		
	Av.	338	364	96.6	100.0
1.00		337	362		
		337	356		
	Av.	337	359	96.3	98.6
1.10		299	230		
		295	226		
	Av.	297	228	85.0	90.0
1.20		226	254		
		226	254		
	Av.	226	254	64.6	70.0
1.30		130	154		
		122	140		
	Av.	126	147	36.0	40.4
1.40		41	64		
		37	50		
	Av.	39	57	11.1	15.7
1.50		3	11		
		3	9		
	Av.	3	10	0.8	2.7
1.60		0	0		
		0	0		
	Av.	0	0	0.0	0.0

TABLE 7

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE AND SODIUM GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent	Sodium Chloride Concentration		Sodium Gluconate Concentration	
Hemolysis	Blood Sample 1	Blood Sample 2	Blood Sample 1	Blood Sample 2
25	0.418	0.428	1.34	1.36
50	0.405	0.418	1.25	1.27
75	0.392	0.402	1.16	1.18

TABLE 8

VALUES OF i FOR SODIUM GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 1	Blood Sample 2
25	2.17	2.19
50	2.24	2.28
75	2.35	2.36
Averages	2.25	2.28

TABLE 9

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN SOLUTIONS CONTAINING 0.2 PER CENT SODIUM CHLORIDE AND VARIOUS CONCENTRATIONS OF SODIUM GLUCONATE

Per Cent Sodium Gluconate	Per Cent Sodium Chloride		Colorimeter Readings		Per Cent Hemolysis	
			Blood Sample 3	Blood Sample 4	Blood Sample 3	Blood Sample 4
0.00	0.20		406	364		
			406	364		
		Av.	406	364	100.5	100.0
0.50	0.20		400	360		
			396	370		
		Av.	398	365	98.5	100.3
0.60	0.20		388	365		
			384	365		
		Av.	386	365	95.5	100.3
0.70	0.20		345	353		
			345	349		
		Av.	345	351	85.4	96.4
0.80	0.20		260	328		
			260	322		
		Av.	260	325	64.4	89.3
0.90	0.20		130	248		
			134	246		
		Av.	132	247	32.7	67.9
1.00	0.20		48	87		
			46	87		
		Av.	47	87	11.6	23.9
1.10	0.20		0	20		
			0	16		
		Av.	0	18	0.0	4.9

TABLE 10

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 3	Blood Sample 4
25	0.459	0.483
50	0.437	0.472
75	0.417	0.460

TABLE 11

CONCENTRATIONS IN GM./100 ML. OF SODIUM GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 3	Blood Sample 4
25	0.928	0.998
50	0.848	0.947
75	0.760	0.878

TABLE 12

VALUES OF i FOR SODIUM GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 3	Blood Sample 4
25	1.94	1.97
50	1.94	1.99
75	2.03	2.06
Averages	1.97	2.01

TABLE 13

PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN SODIUM GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

Per Cent Sodium Gluconate		Colorimeter Readings		Per Cent Hemolysis	
		Blood Sample 30	Blood Sample 31	Blood Sample 30	Blood Sample 31
0.80		474	491		
		484	475		
	Av.	479	483	97.6	96.4
0.90		478	475		
		478	475		
	Av.	478	475	97.4	94.8
1.00		439	469		
		433	457		
	Av.	436	463	88.8	92.4
1.10		292	202		
		296	194		
	Av.	294	198	59.9	39.5
1.20		147	89		
		125	89		
	Av.	136	89	27.7	17.8
1.30		28	40		
		28	40		
	Av.	28	40	5.7	8.0
1.40		0	10		
		0	12		
	Av.	0	11	0.0	2.2
1.50		...	0		
		...	0		
	Av.	...	0	....	0.0

TABLE 14

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE AND SODIUM GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Sodium Chloride Concentration		Sodium Gluconate Concentration	
	Blood Sample 30	Blood Sample 31	Blood Sample 30	Blood Sample 31
25	0.403	0.388	1.20	1.16
50	0.384	0.373	1.13	1.10
75	0.371	0.365	1.06	1.05

TABLE 15

VALUES OF $\underline{i}$ FOR SODIUM GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 30	Blood Sample 31
25	2.33	2.32
50	2.36	2.35
75	2.43	2.41
Averages	2.37	2.36

TABLE 16

PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN SOLUTIONS CONTAINING 0.2 PER CENT SODIUM CHLORIDE AND VARIOUS CONCENTRATIONS OF SODIUM GLUCONATE

Per Cent Sodium Gluconate	Per Cent Sodium Chloride		Colorimeter Readings		Per Cent Hemolysis	
			Blood Sample 32	Blood Sample 33	Blood Sample 32	Blood Sample 33
0.00	0.20		496	424		
			496	424		
		Av.	496	424	100.0	100.0
0.40	0.20		467	399		
			463	395		
		Av.	465	397	93.8	93.6
0.50	0.20		435	380		
			435	370		
		Av.	435	375	87.7	88.4
0.60	0.20		388	220		
			382	228		
		Av.	385	224	77.6	52.8
0.70	0.20		221	59		
			221	53		
		Av.	221	56	44.6	13.2
0.80	0.20		60	0		
			56	0		
		Av.	58	0	11.7	0.0
0.90	0.20		7	0		
			7	0		
		Av.	7	0	1.4	0.0
1.00	0.20		0	0		
			0	0		
		Av.	0	0	0.0	0.0

TABLE 17

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 32	Blood Sample 33
25	0.414	0.396
50	0.403	0.381
75	0.384	0.368

TABLE 18

CONCENTRATIONS IN GM./100 ML. OF SODIUM GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 32	Blood Sample 33
25	0.757	0.660
50	0.685	0.605
75	0.595	0.550

TABLE 19

VALUES OF i FOR SODIUM GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 32	Blood Sample 33
25	1.96	2.06
50	2.05	2.08
75	2.15	2.12
Averages	2.05	2.09

TABLE 20

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN POTASSIUM GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

Per Cent Potassium Gluconate		Colorimeter Readings		Per Cent Hemolysis	
		Blood Sample 5	Blood Sample 6	Blood Sample 5	Blood Sample 6
1.00		286	332		
		286	336		
	Av.	286	334	98.0	101.5
1.10		237	295		
		241	295		
	Av.	239	295	82.0	89.7
1.20		191	274		
		187	278		
	Av.	189	276	64.7	83.9
1.30		124	204		
		140	210		
	Av.	132	207	45.2	62.9
1.40		92	110		
		88	100		
	Av.	90	105	30.8	31.9
1.50		50	20		
		44	18		
	Av.	47	19	16.1	5.8
1.60		30	0		
		22	0		
	Av.	26	0	8.9	0.0
1.70		9	0		
		9	0		
	Av.	9	0	3.1	0.0
1.80		0	0		
		0	0		
	Av.	0	0	0.0	0.0

TABLE 21

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE AND POTASSIUM GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent Hemolysis	Sodium Chloride Concentration		Potassium Gluconate Concentration	
	Blood Sample 5	Blood Sample 6	Blood Sample 5	Blood Sample 6
25	0.427	0.412	1.43	1.42
50	0.388	0.398	1.28	1.35
75	0.364	0.386	1.14	1.25

TABLE 22

VALUES OF *i* FOR POTASSIUM GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 5	Blood Sample 6
25	2.22	2.16
50	2.26	2.21
75	2.37	2.30
Averages	2.28	2.22

TABLE 23

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN SOLUTIONS CONTAINING 0.2 PER CENT SODIUM CHLORIDE AND VARIOUS CONCENTRATIONS OF POTASSIUM GLUCONATE

Per Cent Potassium Gluconate	Per Cent Sodium Chloride		Colorimeter Readings		Per Cent Hemolysis	
			Blood Sample 7	Blood Sample 8	Blood Sample 7	Blood Sample 8
0.00	0.20		406	365		
			406	363		
		Av.	406	364	100.5	100.0
0.50	0.20		405	366		
			411	366		
		Av.	408	366	101.0	100.6
0.60	0.20		390	351		
			388	359		
		Av.	389	355	96.3	97.5
0.70	0.20		350	351		
			358	357		
		Av.	354	354	87.6	97.3
0.80	0.20		289	350		
			289	344		
		Av.	289	347	71.5	95.3
0.90	0.20		169	298		
			175	302		
		Av.	172	300	42.6	82.4
1.00	0.20		79	179		
			79	173		
		Av.	79	176	19.6	48.4
1.10	0.20		5	23		
			5	21		
		Av.	5	22	1.2	6.0

TABLE 24

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 7	Blood Sample 8
25	0.459	0.483
50	0.437	0.472
75	0.417	0.460

TABLE 25

CONCENTRATIONS IN GM./100 ML. OF POTASSIUM GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 7	Blood Sample 8
25	0.973	1.05
50	0.883	0.996
75	0.780	0.928

TABLE 26

VALUES OF i FOR POTASSIUM GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 7	Blood Sample 8
25	1.98	2.01
50	2.05	2.08
75	2.07	2.08
Averages	2.03	2.06

TABLE 27

PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN POTASSIUM GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

Per Cent Potassium Gluconate		Colorimeter Readings		Per Cent Hemolysis	
		Blood Sample 34	Blood Sample 35	Blood Sample 34	Blood Sample 35
0.80		449	478		
		449	470		
	Av.	449	474	98.0	99.6
1.00		439	428		
		439	424		
	Av.	439	426	95.9	89.5
1.10		418	366		
		414	374		
	Av.	416	370	91.0	77.7
1.20		316	315		
		336	321		
	Av.	326	318	71.2	66.8
1.30		152	191		
		146	191		
	Av.	149	191	32.5	40.1
1.40		57	75		
		57	69		
	Av.	57	72	12.5	15.1
1.50		11	12		
		11	12		
	Av.	11	12	2.4	2.5
1.60		0	0		
		0	0		
	Av.	0	0	0.0	0.0

TABLE 28

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE AND POTASSIUM GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Sodium Chloride Concentration		Potassium Gluconate Concentration	
	Blood Sample 34	Blood Sample 35	Blood Sample 34	Blood Sample 35
25	0.398	0.399	1.33	1.36
50	0.385	0.382	1.25	1.27
75	0.372	0.363	1.19	1.14

TABLE 29

VALUES OF *i* FOR POTASSIUM GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 34	Blood Sample 35
25	2.23	2.18
50	2.30	2.25
75	2.33	2.38
Averages	2.29	2.27

TABLE 30

PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN SOLUTIONS CONTAINING 0.2 PER CENT SODIUM CHLORIDE AND VARIOUS CONCENTRATIONS OF POTASSIUM GLUCONATE

Per Cent Potassium Gluconate	Per Cent Sodium Chloride		Colorimeter Readings		Per Cent Hemolysis	
			Blood Sample 36	Blood Sample 37	Blood Sample 36	Blood Sample 37
0.00	0.20		497	420		
			495	428		
		Av.	496	424	100.0	100.0
0.40	0.20		475	410		
			467	406		
		Av.	471	408	95.0	96.2
0.50	0.20		471	370		
			467	380		
		Av.	469	375	94.6	88.4
0.60	0.20		410	292		
			404	278		
		Av.	407	285	82.1	67.2
0.70	0.20		250	89		
			270	89		
		Av.	260	89	54.2	21.0
0.80	0.20		105	22		
			105	18		
		Av.	105	20	21.2	4.7
0.90	0.20		19	0		
			17	0		
		Av.	18	0	3.6	0.0
1.00	0.20		0	0		
			0	0		
		Av.	0	0	0.0	0.0

TABLE 31

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 36	Blood Sample 37
25	0.414	0.396
50	0.403	0.381
75	0.384	0.368

TABLE 32

CONCENTRATIONS IN GM./100 ML. OF POTASSIUM GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 36	Blood Sample 37
25	0.783	0.690
50	0.708	0.637
75	0.628	0.578

TABLE 33

VALUES OF i FOR POTASSIUM GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 36	Blood Sample 37
25	2.04	2.12
50	2.14	2.12
75	2.18	2.17
Averages	2.12	2.14

TABLE 34

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN MAGNESIUM GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

Per Cent Magnesium Gluconate		Colorimeter Readings		Per Cent Hemolysis	
		Blood Sample 9	Blood Sample 10	Blood Sample 9	Blood Sample 10
1.00		366	327		
		364	329		
	Av.	365	328	100.6	102.2
1.50		346	328		
		346	312		
	Av.	346	320	95.3	99.7
1.75		...	279		
		...	273		
	Av.	...	276	....	86.0
2.00		193	153		
		191	161		
	Av.	192	157	52.9	48.9
2.25		104	39		
		96	43		
	Av.	100	41	27.5	12.8
2.50		80	15		
		72	15		
	Av.	76	15	20.9	4.7

TABLE 35

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE AND MAGNESIUM GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent Hemolysis	Sodium Chloride Concentration		Magnesium Gluconate Concentration	
	Blood Sample 9	Blood Sample 10	Blood Sample 9	Blood Sample 10
25	0.416	0.419	2.32	2.14
50	0.399	0.403	2.02	1.99
75	0.380	0.386	1.84	1.84

TABLE 36

VALUES OF i FOR MAGNESIUM GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 9	Blood Sample 10
25	2.37	2.58
50	2.61	2.67
75	2.72	2.77
Averages	2.57	2.67

TABLE 37

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN SOLUTIONS CONTAINING 0.2 PER CENT SODIUM CHLORIDE AND VARIOUS CONCENTRATIONS OF MAGNESIUM GLUCONATE

Per Cent Magnesium Gluconate	Per Cent Sodium Chloride		Colorimeter Readings		Per Cent Hemolysis	
			Blood Sample 11	Blood Sample 12	Blood Sample 11	Blood Sample 12
0.000	0.20		273	299		
			271	295		
		Av.	272	297	100.0	99.0
0.250	0.20		276	300		
			276	294		
		Av.	276	297	101.5	99.0
0.500	0.20		265	296		
			257	296		
		Av.	261	296	96.0	98.7
0.750	0.20		217	287		
			215	273		
		Av.	216	280	79.4	93.4
0.875	0.20		...	226		
			...	226		
		Av.	...	226	....	75.3
1.000	0.20		76	128		
			76	124		
		Av.	76	126	27.9	42.0
1.125	0.20		...	89		
			...	85		
		Av.	...	87	....	29.0
1.250	0.20		9	7		
			5	7		
		Av.	7	7	2.6	2.3
1.500	0.20		0	0		
			0	0		
		Av.	0	0	0.0	0.0

TABLE 38

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 11	Blood Sample 12
25	0.430	0.450
50	0.415	0.435
75	0.394	0.418

TABLE 39

CONCENTRATIONS IN GM./100 ML. OF MAGNESIUM GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 11	Blood Sample 12
25	1.03	1.07
50	0.889	0.975
75	0.775	0.878

TABLE 40

VALUES OF i FOR MAGNESIUM GLUCONATE AT CONCENTRATIONS
CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF
RABBIT ERYTHROCYTES IN THE PRESENCE OF
0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 11	Blood Sample 12
25	2.96	3.10
50	3.19	3.19
75	3.31	3.28
Averages	3.15	3.19

TABLE 41

PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN MAGNESIUM GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

Per Cent Magnesium Gluconate		Colorimeter Readings of Blood Sample			Per Cent Hemolysis of Blood Sample		
		38	39	40	38	39	40
0.50		406	445	470			
		402	441	470			
	Av.	404	443	470	100.3	99.1	95.1
0.75		394	435	458			
		386	435	454			
	Av.	390	435	456	96.8	97.3	92.3
1.00		307	340	388			
		327	352	380			
	Av.	317	346	384	78.7	77.4	77.7
1.25		192	244	233			
		192	240	233			
	Av.	192	242	233	47.6	54.1	47.2
1.50		133	160	150			
		129	170	154			
	Av.	131	165	152	32.5	36.9	30.8
1.75		65	72	44			
		61	76	46			
	Av.	63	74	45	15.6	16.6	9.1
2.00		0	5	7			
		0	5	7			
	Av.	0	5	7	0.0	1.1	1.4
2.25		0	0	0			
		0	0	0			
	Av.	0	0	0	0.0	0.0	0.0

TABLE 42

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE AND MAGNESIUM GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Sodium Chloride Concentration and Blood Sample			Magnesium Gluconate Concentration and Blood Sample		
	38	39	40	38	39	40
25	0.395	0.393	0.405	1.55	1.63	1.51
50	0.381	0.380	0.392	1.28	1.30	1.28
75	0.370	0.363	0.381	1.05	1.05	1.03

TABLE 43

VALUES OF *i* FOR MAGNESIUM GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 38	Blood Sample 39	Blood Sample 40
25	3.36	3.18	3.53
50	3.92	3.86	4.03
75	4.64	4.56	4.87
Averages	3.97	3.87	4.14

TABLE 44

PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN SOLUTIONS CONTAINING 0.2 PER CENT SODIUM CHLORIDE AND VARIOUS CONCENTRATIONS OF MAGNESIUM GLUCONATE

Per Cent Magnesium Gluconate	Per Cent Sodium Chloride		Colorimeter Readings		Per Cent Hemolysis	
			Blood Sample 41	Blood Sample 42	Blood Sample 41	Blood Sample 42
0.000	0.20		482	489		
			480	481		
		Av.	481	485	100.0	97.8
0.375	0.20		462	487		
			462	483		
		Av.	462	485	96.1	97.8
0.500	0.20		446	481		
			442	477		
		Av.	444	479	92.3	96.6
0.625	0.20		398	450		
			389	444		
		Av.	393	447	81.7	90.1
0.750	0.20		144	366		
			142	362		
		Av.	143	364	29.7	73.4
0.875	0.20		69	241		
			69	241		
		Av.	69	241	14.4	48.6
1.000	0.20		7	79		
			3	73		
		Av.	5	76	1.0	15.3
1.250	0.20		0	4		
			0	4		
		Av.	0	4	0.0	0.8

TABLE 45

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 41	Blood Sample 42
25	0.403	0.447
50	0.390	0.427
75	0.378	0.406

TABLE 46

CONCENTRATIONS IN GM./100 ML. OF MAGNESIUM GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 41	Blood Sample 42
25	0.819	0.950
50	0.750	0.864
75	0.669	0.750

TABLE 47

VALUES OF i FOR MAGNESIUM GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 41	Blood Sample 42
25	3.26	3.43
50	3.34	3.46
75	3.51	3.62
Averages	3.37	3.50

TABLE 48

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN CALCIUM GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

Per Cent Calcium Gluconate		Colorimeter Readings		Per Cent Hemolysis	
		Blood Sample 13	Blood Sample 14	Blood Sample 13	Blood Sample 14
1.70		282	224		
		298	224		
	Av.	290	224	94.8	100.0
1.80		276	217		
		266	213		
	Av.	271	215	88.6	96.0
2.00		259	185		
		259	179		
	Av.	259	182	84.6	81.3
2.10		236	156		
		236	154		
	Av.	236	155	77.1	69.2
2.20		224	120		
		220	126		
	Av.	222	123	72.6	54.9
2.30		194	84		
		188	98		
	Av.	191	91	62.4	40.6
2.40		183	54		
		177	64		
	Av.	180	59	58.8	26.3
2.50		140	52		
		140	44		
	Av.	140	48	45.8	21.4
2.60		102	28		
		108	26		
	Av.	105	27	34.3	12.1
2.80		63	0		
		59	0		
	Av.	61	0	19.9	0.0

TABLE 49

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE AND CALCIUM GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent Hemolysis	Sodium Chloride Concentration		Calcium Gluconate Concentration	
	Blood Sample 13	Blood Sample 14	Blood Sample 13	Blood Sample 14
25	0.466	0.410	2.73	2.43
50	0.425	0.395	2.45	2.24
75	0.395	0.378	2.16	2.06

TABLE 50

VALUES OF i FOR CALCIUM GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 13	Blood Sample 14
25	2.34	2.31
50	2.38	2.42
75	2.50	2.51
Averages	2.41	2.41

TABLE 51

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN SOLUTIONS CONTAINING 0.2 PER CENT SODIUM CHLORIDE AND VARIOUS CONCENTRATIONS OF CALCIUM GLUCONATE

Per Cent Calcium Gluconate	Per Cent Sodium Chloride		Colorimeter Readings of Blood Sample			Per Cent Hemolysis of Blood Sample		
			15	16	17	15	16	17
0.00	0.20		300	388	254			
			302	388	254			
		Av.	301	388	254	100.3	100.2	100.0
0.70	0.20		...	340	220			
			...	338	224			
		Av.	...	339	222	....	88.0	87.4
0.80	0.20		299	280	210			
			309	272	212			
		Av.	304	276	211	101.4	71.7	83.1
0.90	0.20		...	...	160			
			...	...	160			
		Av.	...	...	160	....	....	63.0
1.00	0.20		241	119	120			
			235	109	114			
		Av.	238	114	117	79.3	29.6	46.1
1.20	0.20		125	41	28			
			121	39	24			
		Av.	123	40	26	41.0	10.4	10.2
1.40	0.20		61	14	2			
			55	14	2			
		Av.	58	14	2	19.3	3.6	0.8
1.60	0.20		20	9	0			
			24	11	0			
		Av.	22	10	0	7.3	2.6	0.0

TABLE 52

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 15	Blood Sample 16	Blood Sample 17
25	0.473	0.422	0.428
50	0.450	0.399	0.410
75	0.427	0.382	0.394

TABLE 53

CONCENTRATIONS IN GM./100 ML. OF CALCIUM GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 15	Blood Sample 16	Blood Sample 17
25	1.32	1.03	1.09
50	1.16	0.905	0.990
75	1.02	0.785	0.850

TABLE 54

VALUES OF i FOR CALCIUM GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 15	Blood Sample 16	Blood Sample 17
25	2.84	2.95	2.87
50	2.96	3.01	2.91
75	3.04	3.18	3.13
Averages	2.95	3.05	2.97

TABLE 55

PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN CALCIUM GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

Per Cent Calcium Gluconate		Colorimeter Readings		Per Cent Hemolysis	
		Blood Sample 43	Blood Sample 44	Blood Sample 43	Blood Sample 44
1.50		443	400		
		439	426		
	Av.	441	413	94.0	91.2
1.60		425	372		
		425	350		
	Av.	425	361	90.6	79.7
1.70		400	320		
		392	316		
	Av.	396	318	84.5	70.2
1.80		324	280		
		340	276		
	Av.	332	278	70.8	61.4
1.90		300	238		
		300	234		
	Av.	300	236	64.0	52.1
2.00		238	148		
		224	140		
	Av.	231	144	49.3	31.8
2.10		181	103		
		179	103		
	Av.	180	103	38.4	22.7
2.20		100	74		
		104	54		
	Av.	102	64	21.8	14.1
2.30		56	17		
		56	9		
	Av.	56	13	12.0	2.9
2.40		30	0		
		26	0		
	Av.	28	0	6.0	0.0

TABLE 56

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE AND CALCIUM GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Sodium Chloride Concentration		Calcium Gluconate Concentration	
	Blood Sample 43	Blood Sample 44	Blood Sample 43	Blood Sample 44
25	0.408	0.387	2.18	2.19
50	0.395	0.378	2.00	1.90
75	0.385	0.365	1.81	1.68

TABLE 57

VALUES OF *i* FOR CALCIUM GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 43	Blood Sample 44
25	2.56	2.52
50	2.71	2.73
75	2.92	2.98
Averages	2.73	2.74

TABLE 58

PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN SOLUTIONS CONTAINING 0.2 PER CENT SODIUM CHLORIDE AND VARIOUS CONCENTRATIONS OF CALCIUM GLUCONATE

Per Cent Calcium Gluconate	Per Cent Sodium Chloride		Colorimeter Readings		Per Cent Hemolysis	
			Blood Sample 45	Blood Sample 46	Blood Sample 45	Blood Sample 46
0.00	0.20		455	453		
			453	455		
		Av.	454	454	99.8	99.4
0.40	0.20		...	432		
			...	440		
		Av.	...	436	....	95.4
0.50	0.20		409	420		
			405	430		
		Av.	407	425	89.5	93.0
0.80	0.20		336	369		
			332	363		
		Av.	334	366	73.4	80.1
1.00	0.20		184	232		
			178	226		
		Av.	181	229	39.8	50.1
1.10	0.20		...	150		
			...	156		
		Av.	...	153	....	33.5
1.20	0.20		33	69		
			35	61		
		Av.	34	65	7.5	14.2
1.40	0.20		0	21		
			4	19		
		Av.	2	20	0.4	4.4
1.60	0.20		0	0		
			0	0		
		Av.	0	0	0.0	0.0

TABLE 59

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 45	Blood Sample 46
25	0.390	0.403
50	0.377	0.389
75	0.368	0.376

TABLE 60

CONCENTRATIONS IN GM./100 ML. OF CALCIUM GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 45	Blood Sample 46
25	1.07	1.17
50	0.951	0.998
75	0.787	0.842

TABLE 61

VALUES OF *i* FOR CALCIUM GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 45	Blood Sample 46
25	2.43	2.39
50	2.55	2.58
75	2.92	2.86
Averages	2.63	2.61

TABLE 62

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN MANGANESE(II) GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

Per Cent Manganese(II) Gluconate		Blood Sample 18		
		Colorimeter Readings	Average Readings Minus Readings of Manganese(II) Gluconate Blanks	Per Cent Hemolysis
1.80		352	349	
		346	- 8	
	Av.	349	341	99.7
1.90		327	327	
		327	- 8	
	Av.	327	319	93.3
2.00		312	309	
		306	- 9	
	Av.	309	300	87.7
2.10		300	298	
		296	- 11	
	Av.	298	287	83.9
2.20		265	265	
		265	- 15	
	Av.	265	250	73.1
2.40		151	147	
		143	- 15	
	Av.	147	132	38.6
2.60		50	50	
		50	- 17	
	Av.	50	33	9.7
2.80		34	31	
		28	- 18	
	Av.	31	13	3.8
3.00		28	28	
		28	- 22	
	Av.	28	6	1.8

TABLE 63

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN MANGANESE(II) GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

Per Cent Manganese(II) Gluconate		Blood Sample 19		
		Colorimeter Readings	Average Readings Minus Readings of Manganese(II) Gluconate Blanks	Per Cent Hemolysis
1.80		346	342	
		338	- 7	
	Av.	342	335	91.5
1.90		320	320	
		320	- 8	
	Av.	320	312	85.2
2.00		241	238	
		235	- 9	
	Av.	238	229	62.6
2.10		220	219	
		218	- 10	
	Av.	219	209	57.1
2.20		151	151	
		151	- 16	
	Av.	151	135	36.9
2.40		78	74	
		70	- 17	
	Av.	74	57	15.6
2.60		36	33	
		30	- 19	
	Av.	33	14	3.8
2.80		21	21	
		21	- 20	
	Av.	21	1	0.3
3.00		24	25	
		26	- 24	
	Av.	25	1	0.3

TABLE 64

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE AND MANGANESE(II) GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent Hemolysis	Sodium Chloride Concentration		Manganese(II) Gluconate Concentration	
	Blood Sample 18	Blood Sample 19	Blood Sample 18	Blood Sample 19
25	0.435	0.404	2.50	2.30
50	0.419	0.384	2.33	2.14
75	0.404	0.368	2.19	1.98

TABLE 65

VALUES OF i FOR MANGANESE(II) GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 18	Blood Sample 19
25	2.47	2.50
50	2.55	2.54
75	2.61	2.63
Averages	2.54	2.56

TABLE 66

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN SOLUTIONS CONTAINING 0.2 PER CENT SODIUM CHLORIDE AND VARIOUS CONCENTRATIONS OF MANGANESE(II) GLUCONATE

		Blood Sample 20		
Per Cent Manganese(II) Gluconate	Per Cent Sodium Chloride	Colorimeter Readings	Average Readings Minus Readings Manganese(II) Gluconate Blanks	Per Cent Hemolysis
0.00	0.20	343	...	
		339	...	
	Av.	341	...	99.7
0.80	0.20	358	356	
		354	- 9	
	Av.	356	347	101.5
1.00	0.20	317	314	
		311	- 9	
	Av.	314	305	89.2
1.10	0.20	299	294	
		289	- 9	
	Av.	294	285	83.3
1.20	0.20	267	263	
		259	- 9	
	Av.	263	254	74.3
1.40	0.20	231	231	
		231	- 9	
	Av.	231	222	64.9
1.60	0.20	110	108	
		106	- 11	
	Av.	108	97	28.4
1.80	0.20	63	54	
		45	- 12	
	Av.	54	42	12.3
2.00	0.20	25	25	
		25	- 16	
	Av.	25	9	2.6

TABLE 67

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN SOLUTIONS CONTAINING 0.2 PER CENT SODIUM CHLORIDE AND VARIOUS CONCENTRATIONS OF MANGANESE(II) GLUCONATE

		Blood Sample 21		
Per Cent Manganese(II) Gluconate	Per Cent Sodium Chloride	Colorimeter Readings	Average Readings Minus Readings Manganese(II) Gluconate Blanks	Per Cent Hemolysis
0.00	0.20	368	...	
		360	...	
		Av. 364	...	99.5
0.80	0.20	381	381	
		381	- 10	
		Av. 381	371	101.4
1.00	0.20	301	302	
		303	- 13	
		Av. 302	289	79.0
1.10	0.20	252	256	
		260	- 13	
		Av. 256	243	66.4
1.20	0.20	209	206	
		203	- 22	
		Av. 206	184	50.3
1.40	0.20	158	158	
		158	- 22	
		Av. 158	136	37.2
1.60	0.20	67	63	
		59	- 22	
		Av. 63	41	11.2
1.80	0.20	41	37	
		33	- 23	
		Av. 37	14	3.8
2.00	0.20	32	32	
		32	- 24	
		Av. 32	8	2.2

TABLE 68

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 20	Blood Sample 21
25	0.435	0.404
50	0.419	0.384
75	0.404	0.368

TABLE 69

CONCENTRATIONS IN GM./100 ML. OF MANGANESE(II) GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 20	Blood Sample 21
25	1.63	1.41
50	1.42	1.20
75	1.19	1.04

TABLE 70

VALUES OF *i* FOR MANGANESE(II) GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 20	Blood Sample 21
25	2.04	2.05
50	2.19	2.17
75	2.43	2.29
Averages	2.22	2.17

TABLE 71

PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN MANGANESE(II) GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

Per Cent Manganese(II) Gluconate		Blood Sample 47		
		Colorimeter Readings	Average Readings Minus Readings of Manganese(II) Gluconate Blanks	Per Cent Hemolysis
1.60		467	465	
		463	- 7	
	Av.	465	458	99.1
1.80		398	394	
		390	- 8	
	Av.	394	386	83.5
2.00		185	181	
		177	- 8	
	Av.	181	173	37.5
2.20		46	44	
		42	- 15	
	Av.	44	29	6.3
2.40		25	23	
		21	- 14	
	Av.	23	9	1.9
2.60		22	22	
		22	- 15	
	Av.	22	7	1.5
2.80		19	20	
		21	- 18	
	Av.	20	2	0.4

TABLE 72

PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN MANGANESE(II) GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

		Blood Sample 48		
Per Cent Manganese(II) Gluconate		Colorimeter Readings	Average Readings Minus Readings of Manganese(II) Gluconate Blanks	Per Cent Hemolysis
1.60		480	483	
		486	- 0	
	Av.	483	483	100.4
1.70		472	469	
		466	- 0	
	Av.	469	469	97.5
1.80		418	412	
		406	- 0	
	Av.	412	412	85.7
1.90		269	267	
		265	- 1	
	Av.	267	266	55.3
2.00		168	167	
		165	- 1	
	Av.	167	166	34.5
2.10		94	90	
		86	- 1	
	Av.	90	89	18.5
2.20		33	32	
		31	- 4	
	Av.	32	28	5.8
2.40		12	12	
		12	- 6	
	Av.	12	6	1.2

TABLE 73

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE AND MANGANESE(II) GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Sodium Chloride Concentration		Manganese(II) Gluconate Concentration	
	Blood Sample 47	Blood Sample 48	Blood Sample 47	Blood Sample 48
25	0.384	0.380	2.05	2.05
50	0.372	0.369	1.95	1.94
75	0.364	0.354	1.85	1.84

TABLE 74

VALUES OF *i* FOR MANGANESE(II) GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 47	Blood Sample 48
25	2.65	2.64
50	2.70	2.70
75	2.79	2.73
Averages	2.71	2.69

TABLE 75

PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN SOLUTIONS CONTAINING 0.2 PER CENT SODIUM CHLORIDE AND VARIOUS CONCENTRATIONS OF MANGANESE(II) GLUCONATE

Per Cent Manganese(II) Gluconate	Per Cent Sodium Chloride	Blood Sample 49		
		Colorimeter Readings	Average Readings Minus Readings Manganese(II) Gluconate Blanks	Per Cent Hemolysis
0.00	0.20	464	...	
		466	...	
		Av. 465	...	100.6
0.50	0.20	474	475	
		476	- 0	
		Av. 475	475	102.8
0.80	0.20	460	456	
		452	- 0	
		Av. 456	456	98.7
1.00	0.20	308	300	
		292	- 0	
		Av. 300	300	64.9
1.20	0.20	94	92	
		90	- 3	
		Av. 92	89	19.3
1.40	0.20	26	27	
		28	- 4	
		Av. 27	23	5.0
1.60	0.20	16	16	
		16	- 7	
		Av. 16	9	2.0
1.80	0.20	11	9	
		7	- 9	
		Av. 9	0	0.0

TABLE 76

PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN SOLUTIONS CONTAINING 0.2 PER CENT SODIUM CHLORIDE AND VARIOUS CONCENTRATIONS OF MANGANESE(II) GLUCONATE

Per Cent Manganese(II) Gluconate	Per Cent Sodium Chloride		Blood Sample 50		
			Colorimeter Readings	Average Readings Minus Readings Manganese(II) Gluconate Blanks	Per Cent Hemolysis
0.00	0.20		480	480	
			480	- 0	
		Av.	480	480	99.8
0.50	0.20		487	484	
			481	- 0	
		Av.	484	484	100.6
0.80	0.20		392	394	
			396	- 0	
		Av.	394	394	81.9
0.90	0.20		326	328	
			330	- 0	
		Av.	328	328	68.2
1.00	0.20		247	242	
			237	- 0	
		Av.	242	242	50.3
1.10	0.20		128	130	
			132	- 0	
		Av.	130	130	27.0
1.20	0.20		50	48	
			46	- 3	
		Av.	48	45	9.4
1.30	0.20		12	12	
			12	- 4	
		Av.	12	8	1.6
1.40	0.20		6	5	
			4	- 4	
		Av.	5	1	0.2

TABLE 77

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 49	Blood Sample 50
25	0.384	0.380
50	0.372	0.369
75	0.364	0.354

TABLE 78

CONCENTRATIONS IN GM./100 ML. OF MANGANESE(II) GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 49	Blood Sample 50
25	1.16	1.11
50	1.04	1.00
75	0.970	0.860

TABLE 79

VALUES OF *i* FOR MANGANESE(II) GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 49	Blood Sample 50
25	2.25	2.30
50	2.34	2.40
75	2.40	2.54
Averages	2.33	2.41

TABLE 80

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN IRON(II) GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

		Blood Sample 22		
Per Cent Iron(II) Gluconate		Colorimeter Readings	Average Readings Minus Readings of Iron(II) Gluconate Blanks	Per Cent Hemolysis
2.50		406	398	
		390	- 61	
	Av.	398	337	92.1
3.50		425	419	
		413	- 89	
	Av.	419	330	90.2
3.70		429	426	
		423	- 98	
	Av.	426	328	89.7
4.00		435	435	
		435	-108	
	Av.	435	327	89.6
4.20		430	428	
		426	-115	
	Av.	428	313	85.6
4.60		437	441	
		445	-129	
	Av.	441	312	85.4
4.80		426	426	
		426	-136	
	Av.	426	290	79.3
5.00		385	382	
		379	-147	
	Av.	382	235	64.2
5.50		266	262	
		258	-165	
	Av.	262	97	26.5

TABLE 81

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN IRON(II) GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

Per Cent Iron(II) Gluconate		Blood Sample 23		
		Colorimeter Readings	Average Readings Minus Readings of Iron(II) Gluconate Blanks	Per Cent Hemolysis
2.50		390	387	
		384	- 71	
	Av.	387	316	90.6
3.50		415	413	
		411	-105	
	Av.	413	308	88.3
3.70		414	414	
		414	-117	
	Av.	414	297	85.1
4.00		421	420	
		419	-124	
	Av.	420	296	84.9
4.20		402	401	
		400	-135	
	Av.	401	266	76.2
4.40		376	370	
		364	-137	
	Av.	370	233	66.8
4.60		330	327	
		324	-155	
	Av.	327	172	49.3
4.80		316	316	
		316	-160	
	Av.	316	156	44.7
5.00		262	264	
		266	-154	
	Av.	264	110	31.6
5.50		236	234	
		232	-181	
	Av.	234	53	15.2

TABLE 82

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE AND IRON(II) GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent Hemolysis	Sodium Chloride Concentration		Iron(II) Gluconate Concentration	
	Blood Sample 22	Blood Sample 23	Blood Sample 22	Blood Sample 23
25	0.492	0.424	5.53	5.15
50	0.477	0.409	5.19	4.67
75	0.461	0.377	4.83	4.22

TABLE 83

VALUES OF *i* FOR IRON(II) GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 22	Blood Sample 23
25	1.27	1.17
50	1.31	1.25
75	1.36	1.27
Averages	1.31	1.23

TABLE 84

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN SOLUTIONS CONTAINING 0.2 PER CENT SODIUM CHLORIDE AND VARIOUS CONCENTRATIONS OF IRON(II) GLUCONATE

			Blood Sample 24		
Per Cent Iron(II) Gluconate	Per Cent Sodium Chloride		Colorimeter Readings	Average Readings Minus Readings of Iron(II) Gluconate Blanks	Per Cent Hemolysis
0.00	0.20		337	...	
			337	...	
		Av.	337	...	100.2
0.50[a]	0.20		264	262	
			260	- 0	
		Av.	262	262	78.9
1.00	0.20		277	275	
			273	- 10	
		Av.	275	265	79.8
1.50	0.20		245	242	
			239	- 19	
		Av.	242	223	67.2
1.75	0.20		240	238	
			236	- 24	
		Av.	238	214	64.5
2.00	0.20		268	271	
			274	- 29	
		Av.	271	242	71.4
2.50	0.20		300	301	
			302	- 38	
		Av.	301	263	79.3
3.00	0.20		246	246	
			246	- 49	
		Av.	246	197	59.4
4.00	0.20		134	132	
			130	- 72	
		Av.	132	60	19.9
5.00	0.20		136	133	
			130	- 92	
		Av.	133	41	12.3

[a] - Precipitation was observed in tubes of this concentration.

TABLE 85

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN SOLUTIONS CONTAINING 0.2 PER CENT SODIUM CHLORIDE AND VARIOUS CONCENTRATIONS OF IRON(II) GLUCONATE

			Blood Sample 25		
Per Cent Iron(II) Gluconate	Per Cent Sodium Chloride		Colorimeter Readings	Average Readings Minus Readings of Iron(II) Gluconate Blanks	Per Cent Hemolysis
0.00	0.20		330	...	
			334	...	
		Av.	332	...	100.9
1.50	0.20		178	179	
			180	- 23	
		Av.	179	156	47.4
1.75	0.20		178	178	
			178	- 24	
		Av.	178	154	46.9
2.00	0.20		171	169	
			167	- 33	
		Av.	169	136	41.4
2.25	0.20		194	193	
			192	- 39	
		Av.	193	154	46.9
2.50	0.20		218	221	
			223	- 40	
		Av.	221	181	55.1
2.75	0.20		249	248	
			247	- 47	
		Av.	248	201	61.2
3.00	0.20		225	227	
			229	- 52	
		Av.	227	175	53.2
4.00	0.20		127	130	
			133	- 76	
		Av.	130	54	16.4
5.00	0.20		123	121	
			119	- 93	
		Av.	121	28	8.5

TABLE 86

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 24	Blood Sample 25
25	0.445	0.449
50	0.425	0.430
75	0.409	0.405

TABLE 87

CONCENTRATIONS IN GM./100 ML. OF IRON(II) GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 24	Blood Sample 25
25	3.93	3.65
50	3.18	3.05[b] 2.44
75	2.60[a] 2.24 1.14	....[c]

a - The curve drawn for this blood sample intersected the point of 75 per cent hemolysis 3 times.

b - The curve drawn for this blood sample intersected the point of 50 per cent hemolysis 2 times.

c - The curve drawn for this blood sample did not extend to the point of 75 per cent hemolysis.

TABLE 88

VALUES OF i FOR IRON(II) GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 24	Blood Sample 25
25	0.89	0.97
50	1.01	1.20[b]
75	1.69[a]	....[c]
Averages	1.20	1.09

a - Average i value calculated from three concentrations of iron(II) gluconate causing 75 per cent hemolysis of this blood sample.

b - Average i value calculated from two concentrations of iron(II) gluconate causing 50 per cent hemolysis of this blood sample.

c - No i value was obtainable for this blood sample at 75 per cent hemolysis.

TABLE 89

PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN IRON(II) GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

		Blood Sample 51		
Per Cent Iron(II) Gluconate		Colorimeter Readings	Average Readings Minus Readings of Iron(II) Gluconate Blanks	Per Cent Hemolysis
1.00		297	295	
		293	- 3	
	Av.	295	292	85.5
1.50		307	303	
		299	- 12	
	Av.	303	291	85.1
2.00		299	297	
		295	- 18	
	Av.	297	279	81.6
2.50		292	289	
		286	- 33	
	Av.	289	256	74.9
3.00		213	213	
		213	- 42	
	Av.	213	171	50.0
3.50		133	130	
		127	- 53	
	Av.	130	77	22.5
3.75		98	98	
		98	- 61	
	Av.	98	37	10.8
4.00		88	87	
		86	- 66	
	Av.	87	21	6.1
4.50		100	99	
		98	- 79	
	Av.	99	20	5.9
5.00		107	106	
		105	- 87	
	Av.	106	19	5.5

TABLE 90

PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN IRON(II) GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

Per Cent Iron(II) Gluconate		Blood Sample 52 Colorimeter Readings	Average Readings Minus Readings of Iron(II) Gluconate Blanks	Per Cent Hemolysis
1.00		350 346	348 - 0	
	Av.	348	348	83.5
1.50		342 342	342 - 12	
	Av.	342	330	79.2
2.00		333 329	331 - 15	
	Av.	331	316	75.8
2.50		315 317	316 - 24	
	Av.	316	292	70.0
3.00		200 194	197 - 32	
	Av.	197	165	39.6
3.50		73 73	73 - 52	
	Av.	73	21	5.0
3.75		71 71	71 - 61	
	Av.	71	10	2.4
4.00		73 69	71 - 66	
	Av.	71	5	1.2
4.50		85 77	81 - 76	
	Av.	81	5	1.2
5.00		98 94	96 - 88	
	Av.	96	8	1.9

TABLE 91

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE AND IRON(II) GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Sodium Chloride Concentration		Iron(II) Gluconate Concentration	
	Blood Sample 51	Blood Sample 52	Blood Sample 51	Blood Sample 52
25	0.355	0.328	3.45	3.24
50	0.328	0.322	3.00	2.88
75	0.298	0.306	2.48	2.32

TABLE 92

VALUES OF *i* FOR IRON(II) GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 51	Blood Sample 52
25	1.46	1.44
50	1.56	1.60
75	1.71	1.87
Averages	1.58	1.64

TABLE 93

PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN SOLUTIONS CONTAINING 0.2 PER CENT SODIUM CHLORIDE AND VARIOUS CONCENTRATIONS OF IRON(II) GLUCONATE

			Blood Sample 53		
Per Cent Iron(II) Gluconate	Per Cent Sodium Chloride		Colorimeter Readings	Average Readings Minus Readings of Iron(II) Gluconate Blanks	Per Cent Hemolysis
0.00	0.20		414	...	
			410	...	
		Av.	412	...	96.3
0.50	0.20		302	299	
			296	- 0	
		Av.	299	299	70.0
1.00	0.20		254	251	
			248	- 1	
		Av.	251	250	58.5
1.50	0.20		206	204	
			202	- 6	
		Av.	204	198	46.2
1.75	0.20		217	217	
			217	- 14	
		Av.	217	203	47.5
2.00	0.20		231	227	
			223	- 17	
		Av.	227	210	49.0
2.50	0.20		273	270	
			267	- 29	
		Av.	270	241	56.3
3.00	0.20		189	187	
			185	- 42	
		Av.	187	145	33.8
4.00	0.20		80	79	
			78	- 58	
		Av.	79	21	4.9
5.00	0.20		103	101	
			99	- 84	
		Av.	101	17	4.0

TABLE 94

PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN SOLUTIONS CONTAINING 0.2 PER CENT SODIUM CHLORIDE AND VARIOUS CONCENTRATIONS OF IRON(II) GLUCONATE

			Blood Sample 54		
Per Cent Iron(II) Gluconate	Per Cent Sodium Chloride		Colorimeter Readings	Average Readings Minus Readings of Iron(II) Gluconate Blanks	Per Cent Hemolysis
0.00	0.20		490	...	
			488	...	
		Av.	489	...	100.1
0.50	0.20		420	416	
			412	- 0	
		Av.	416	416	85.5
1.00	0.20		366	362	
			358	- 12	
		Av.	362	350	71.9
1.50	0.20		291	291	
			291	- 20	
		Av.	291	271	55.7
1.75	0.20		242	237	
			232	- 25	
		Av.	237	212	43.5
2.00	0.20		256	262	
			268	- 30	
		Av.	262	232	47.7
2.50	0.20		344	340	
			336	- 43	
		Av.	340	297	61.0
3.00	0.20		227	224	
			221	- 55	
		Av.	224	169	34.7
4.00	0.20		98	97	
			96	- 80	
		Av.	97	17	3.5
5.00	0.20		129	128	
			127	-103	
		Av.	128	25	5.1

TABLE 95

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 53	Blood Sample 54
25	0.353	0.384
50	0.340	0.374
75	0.322	0.361

TABLE 96

CONCENTRATIONS IN GM./100 ML. OF IRON(II) GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 53	Blood Sample 54
25	3.27	3.27
50	2.60[a] 2.07 1.40	2.67[a] 2.14 1.64
75	....[b]	0.894

a - Curves drawn for these blood samples intersected the point of 50 per cent hemolysis 3 times.

b.- Curve drawn for this blood sample did not extend to the point of 75 per cent hemolysis.

TABLE 97

VALUES OF i FOR IRON(II) GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN THE PRESENCE OF 0.2 PER CENT SODIUM CHLORIDE

Per Cent Hemolysis	Blood Sample 53	Blood Sample 54
25	0.67	0.80
50	1.05[a]	1.18[c]
75	....[b]	2.56
Averages	0.86	1.51

a - Average i value calculated from the 3 concentrations of iron(II) gluconate causing 50 per cent hemolysis of this blood sample.

b - No i value was obtainable for this blood sample at 75 per cent hemolysis.

c - Average i value calculated from the 3 concentrations of iron(II) gluconate causing 50 per cent hemolysis of this blood sample.

TABLE 98

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN COBALT(II) GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

		Blood Sample 26		
Per Cent Cobalt(II) Gluconate		Colorimeter Readings[a]	100 Per Cent Hemolysis Reading Minus Average Cobalt(II) Gluconate Readings	Per Cent Hemolysis
0.85		2	309	
		0	- 1	
	Av.	1	308	99.7
1.10		14	309	
		20	- 17	
	Av.	17	292	94.5
1.40		38	309	
		38	- 38	
	Av.	38	271	87.7
1.65		59	309	
		53	- 56	
	Av.	56	253	81.9
1.90		70	309	
		62	- 66	
	Av.	66	243	78.6
2.15		101	309	
		71	- 86	
	Av.	86	223	72.2
2.40		224	309	
		204	-214	
	Av.	214	95	30.7
2.65		300	309	
		282	-291	
	Av.	291	18	5.8
2.90		304	309	
		316	-310	
	Av.	310	- 1	- 0.3

[a] - Colorimeter readings are of solutions resulting from the laking, in 0.1 per cent sodium carbonate solution, of those erythrocytes which failed to hemolyze in the cobalt(II) gluconate solutions.

TABLE 99

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN COBALT(II) GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

		Blood Sample 27		
Per Cent Cobalt(II) Gluconate		Colorimeter Readings[a]	100 Per Cent Hemolysis Reading Minus Average Cobalt(II) Gluconate Readings	Per Cent Hemolysis
0.85		10	346	
		4	- 7	
	Av.	7	339	98.0
1.10		37	346	
		37	- 37	
	Av.	37	309	89.3
1.40		71	346	
		67	- 69	
	Av.	69	277	80.1
2.25		87	346	
		87	- 87	
	Av.	87	259	74.9
2.40		100	346	
		104	-102	
	Av.	102	244	70.5
2.65		188	346	
		172	-180	
	Av.	180	166	48.0
2.90		278	346	
		278	-278	
	Av.	278	68	19.5
3.20		337	346	
		329	-333	
	Av.	333	13	3.8

a - Colorimeter readings are of solutions resulting from the laking, in 0.1 per cent sodium carbonate solution, of those erythrocytes which failed to hemolyze in the cobalt(II) gluconate solutions.

TABLE 100

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE AND COBALT(II) GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent Hemolysis	Sodium Chloride Concentration		Cobalt(II) Gluconate Concentration	
	Blood Sample 26	Blood Sample 27	Blood Sample 26	Blood Sample 27
25	0.389	0.434	2.45	2.88
50	0.377	0.416	2.35	2.64
75	0.361	0.393	2.10	2.24

TABLE 101

VALUES OF *i* FOR COBALT(II) GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 26	Blood Sample 27
25	2.27	2.15
50	2.29	2.25
75	2.46	2.51
Averages	2.34	2.30

TABLE 102

PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN COBALT(II) GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

		Blood Sample 55		
Per Cent Cobalt(II) Gluconate		Colorimeter Readings[a]	100 Per Cent Hemolysis Reading Minus Average Cobalt(II) Gluconate Readings	Per Cent Hemolysis
2.40		39	452	
		39	- 39	
	Av.	39	413	91.4
2.65		111	452	
		109	-110	
	Av.	110	342	75.7
2.90		292	452	
		274	-283	
	Av.	283	169	37.4
3.20		370	452	
		398	-384	
	Av.	384	68	14.0
3.45		445	452	
		439	-442	
	Av.	442	10	2.2

a - Colorimeter readings are of solutions resulting from the laking, in 0.1 sodium carbonate solution, of those erythrocytes which failed to hemolyze in the cobalt(II) gluconate solutions.

TABLE 103

PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN COBALT(II) GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

		Blood Sample 56		
Per Cent Cobalt(II) Gluconate		Colorimeter Readings[a]	100 Per Cent Hemolysis Reading Minus Average Cobalt(II) Gluconate Readings	Per Cent Hemolysis
2.40		43	521	
		57	- 50	
	Av.	50	471	90.4
2.65		237	521	
		247	-242	
	Av.	242	279	53.5
2.90		382	521	
		400	-391	
	Av.	391	130	24.9
3.20		511	521	
		499	-505	
	Av.	505	16	1.1
3.45		523	521	
		519	-521	
	Av.	521	0	0.0

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[a] - Colorimeter readings are of solutions resulting from the laking, in 0.1 per cent sodium carbonate solution, of those erythrocytes which failed to hemolyze in the cobalt(II) gluconate solutions.

TABLE 104

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE AND COBALT(II) GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Sodium Chloride Concentration		Cobalt(II) Gluconate Concentration	
	Blood Sample 55	Blood Sample 56	Blood Sample 55	Blood Sample 56
25	0.423	0.402	3.03	2.92
50	0.413	0.392	2.84	2.70
75	0.407	0.385	2.67	2.53

TABLE 105

VALUES OF *i* FOR COBALT(II) GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 55	Blood Sample 56
25	2.00	1.97
50	2.10	2.07
75	2.18	2.18
Averages	2.09	2.07

TABLE 106

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN ZINC GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

Per Cent Zinc Gluconate		Colorimeter Readings		Per Cent Hemolysis	
		Blood Sample 28	Blood Sample 29	Blood Sample 28	Blood Sample 29
0.0055		350	331		
		356	325		
	Av.	353	328	90.7	83.7
0.0060		340	262		
		320	252		
	Av.	330	257	85.1	65.6
0.0065		235	210		
		233	206		
	Av.	234	208	60.3	53.0
0.0070		210	172		
		214	180		
	Av.	212	176	54.7	45.0
0.0075		...	150		
		...	148		
	Av.	...	149	....	38.0
0.0080		110	128		
		122	120		
	Av.	116	124	29.9	31.6
0.0090		67	72		
		73	80		
	Av.	70	76	18.0	19.4
0.0100		50	45		
		56	45		
	Av.	53	45	13.7	11.5

TABLE 107

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE AND ZINC GLUCONATE CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent Hemolysis	Sodium Chloride Concentration		Zinc Gluconate Concentration	
	Blood Sample 28	Blood Sample 29	Blood Sample 28	Blood Sample 29
25	0.469	0.454	0.00834	0.00855
50	0.444	0.436	0.00704	0.00665
75	0.424	0.423	0.00625	0.00573

TABLE 108

VALUES OF $\underline{i}$ FOR ZINC GLUCONATE AT CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 28	Blood Sample 29
25	816	770
50	915	951
75	984	1070
Averages	905	930

TABLE 109

PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN ZINC GLUCONATE SOLUTIONS OF VARIOUS CONCENTRATIONS

Per Cent Zinc Gluconate		Colorimeter Readings		Per Cent Hemolysis	
		Blood Sample 57	Blood Sample 58	Blood Sample 57	Blood Sample 58
0.0070		412	428		
		402	436		
	Av.	407	432	86.0	88.2
0.0075		410	406		
		402	414		
	Av.	406	410	85.8	83.7
0.0080		386	400		
		380	412		
	Av.	383	406	81.0	82.9
0.0085		384	386		
		380	390		
	Av.	382	388	80.7	79.2
0.0090		326	345		
		310	337		
	Av.	318	341	67.2	70.0
0.0100		262	311		
		262	311		
	Av.	262	311	55.4	63.5
0.0120		206	264		
		194	258		
	Av.	200	261	42.3	53.3
0.0160		152	198		
		158	202		
	Av.	155	200	32.8	40.8
0.0180		...	188		
		...	208		
	Av.	...	198	....	40.4
0.0200		...	198		
		...	196		
	Av.	...	197	....	40.2

TABLE 110

CONCENTRATIONS IN GM./100 ML. OF SODIUM CHLORIDE AND ZINC GLUCONATE CAUSING 50 and 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Sodium Chloride Concentration		Zinc Gluconate Concentration	
	Blood Sample 57	Blood Sample 58	Blood Sample 57	Blood Sample 58
50	0.382	0.380	0.0107	0.0126
75	0.373	0.680	0.00840	0.00848

TABLE 111

VALUES OF *i* FOR ZINC GLUCONATE AT CONCENTRATIONS CAUSING 50 AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES

Per Cent Hemolysis	Blood Sample 57	Blood Sample 58
50	541	457
75	673	658
Averages	607	557

TABLE 112

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN SOLUTIONS 0.1 MOLAR (0.6 PER CENT) IN SODIUM CHLORIDE AND 0.05 MOLAR IN GLUCONATE

Gluconate	Average Colorimeter Readings[a]		Per Cent Hemolysis	
	Blood Sample 12	Blood Sample 29	Blood Sample 12	Blood Sample 29
Sodium	0	0	0.0	0.0
Potassium	0	0	0.0	0.0
Magnesium	0	0	0.0	0.0
Calcium	0	0	0.0	0.0
Manganese(II)[b]	3	6	1.0	1.5
Iron(II)[b]	1	5	0.3	1.3
Copper(II)[c]	...	...	...	...
Cobalt(II)[b]	0	2	0.0	0.5
Zinc	0	1	0.0	0.3

a - Each reading shown is the average reading of two duplicate tubes.

b - Determined by the methods devised for colored solutions.

c - Readings unobtainable due to denaturation of the blood.

TABLE 113

PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES IN SOLUTIONS 0.1 MOLAR (0.6 PER CENT) IN SODIUM CHLORIDE AND 0.05 MOLAR IN GLUCONATE

Gluconate	Average Colorimeter Readings[a]		Per Cent Hemolysis	
	Blood Sample 31	Blood Sample 47	Blood Sample 31	Blood Sample 47
Sodium	0	0	0.0	0.0
Potassium	0	0	0.0	0.0
Magnesium	0	0	0.0	0.0
Calcium	0	0	0.0	0.0
Manganese(II)[b]	23	16	4.6	3.5
Iron(II)[b]	194[c]	134[c]	38.7	29.0
Cobalt(II)[b]	76	30	15.2	6.5
Copper(II)[d]	...	...	....	....
Zinc	23	4	4.6	0.8

a - Each reading shown is the average of two duplicate tubes.

b - Determined by the methods devised for colored solutions.

c - Precipitate of unknown composition observed.

d - Readings unobtainable due to denaturation of blood.

TABLE 114

VALUES OF i, FOR VARIOUS GLUCONATES, CALCULATED FROM CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES[a]

Gluconate	Per Cent Hemolysis			Averages
	25	50	75	
Sodium Gluconate	2.18	2.26	2.36	2.27
Sodium Gluconate[b]	1.96	1.97	2.05	1.99
Potassium Gluconate	2.19	2.24	2.34	2.26
Potassium Gluconate[b]	2.00	2.07	2.08	2.05
Magnesium Gluconate	2.48	2.64	2.76	2.63
Magnesium Gluconate[b]	3.03	3.19	3.30	3.17
Calcium Gluconate	2.32	2.40	2.50	2.41
Calcium Gluconate[b,c]	2.89	2.96	3.12	2.99
Manganese(II) Gluconate	2.48	2.54	2.62	2.55
Manganese(II) Gluconate[b]	2.04	2.18	2.36	2.19
Iron(II) Gluconate	1.22	1.28	1.32	1.27
Iron(II) Gluconate[b]	0.93	1.11	1.69[d]	1.24
Cobalt(II) Gluconate	2.21	2.27	2.48	2.32
Zinc Gluconate	793.	933.	1027.	918.

a - Unless otherwise indicated all i values represent an average of two blood samples.

b - Values obtained in the presence of 0.2 per cent sodium chloride.

c - Average of three blood samples.

d - Result of one blood sample.

TABLE 115

VALUES OF i, FOR VARIOUS GLUCONATES, CALCULATED FROM CONCENTRATIONS CAUSING 25, 50, AND 75 PER CENT HEMOLYSIS OF HUMAN ERYTHROCYTES[a]

Gluconate	Per Cent Hemolysis 25	50	75	Averages
Sodium Gluconate	2.32	2.36	2.42	2.37
Sodium Gluconate[b]	2.01	2.07	2.14	2.07
Potassium Gluconate	2.20	2.28	2.36	2.28
Potassium Gluconate[b]	2.08	2.13	2.18	2.13
Magnesium Gluconate[c]	3.36	3.94	4.69	4.00
Magnesium Gluconate[b]	3.34	3.40	3.56	3.43
Calcium Gluconate	2.54	2.72	2.95	2.74
Calcium Gluconate[b]	2.41	2.56	2.89	2.62
Manganese(II) Gluconate	2.64	2.70	2.76	2.70
Manganese(II) Gluconate[b]	2.28	2.37	2.47	2.37
Iron(II) Gluconate	1.45	1.58	1.79	1.61
Iron(II) Gluconate[b]	0.74	1.12	2.56[d]	1.47
Cobalt(II) Gluconate	1.98	2.08	2.18	2.08
Zinc Gluconate	....	499.	666.	582.

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[a] - Unless otherwise indicated all i values represent an average of two blood samples.

[b] - Values obtained in the presence of 0.2 per cent sodium chloride.

[c] - Average of three blood samples.

[d] - Result of one blood sample.

TABLE 116

pH VALUES OF 2 PER CENT SOLUTIONS OF VARIOUS GLUCONATES BEFORE AND AFTER ADDITION OF RABBIT BLOOD[a]

Gluconate	pH Before The Addition of Blood Sample		pH 45 Minutes After The Addition of Blood Sample[b]	
	17	28	17	28
Sodium	6.98	6.88	7.04	7.00
Potassium	7.02	6.90	7.07	6.98
Magnesium	7.36	7.25	7.50	7.42
Calcium	7.72	7.60	7.80	7.70
Manganese(II)	6.47	6.49	6.51	6.49
Iron(II)	4.55	4.50	4.57	4.55
Cobalt(II)	5.15	5.13	5.15	5.20
Copper(II)	3.95	3.85	3.95	3.88
Zinc	6.40	6.40	6.52	6.44

a - To 10.0 ml. of the gluconate solution, 0.10 ml. of defibrinated blood was added.

b - The pH value of Blood Sample 17 was 7.85 and that of Blood Sample 28 was 7.80.

TABLE 117

PER CENT HEMOLYSIS OF RABBIT ERYTHROCYTES IN SODIUM CHLORIDE SOLUTIONS OF VARIOUS CONCENTRATIONS AND pH

Per Cent Sodium Chloride	pH NaCl Solutions Before Blood Added		Average Colorimeter Readings		Per Cent Hemolysis	
	Blood Sample 17	Blood Sample 28	Blood Sample 17	Blood Sample 28	Blood Sample 17	Blood Sample 28
0.39	5.90	6.12	205	366	80.7	94.3
	5.52	5.08	209	383	82.2	98.8
	5.21	....	216	...	83.3	....
	4.68	....	210	...	82.7	....
	3.98	....	235	...	92.6	....
	3.64	....	243	...	95.8	....
0.40	5.88	6.11	155	361	61.0	93.0
	4.88	5.13	179	360	70.3	92.7
	4.23	4.04	235	368	92.6	94.9
	3.52	3.56	250	379	98.6	97.6
0.41	6.13	6.20	126	350	49.6	90.2
	4.96	4.68	131	350	51.6	90.2
	4.55	4.22	160	371	62.9	95.6
	4.26	3.51	201	374	79.0	96.3
	3.92	....	223	...	87.8	....
	3.51	....	248	...	97.5	....
0.42	6.06	6.20	81	310	31.9	79.9
	5.50	5.62	89	326	35.1	84.1
	4.70	4.45	110	350	43.3	90.2
	4.10	4.00	194	366	76.5	94.4
	3.50	3.60	246	379	96.9	97.6

(Table continued on following page)

TABLE 117 (continued)

Per Cent Sodium Chloride	pH NaCl Solutions Before Blood Added		Average Colorimeter Readings		Per Cent Hemolysis	
	Blood Sample 17	Blood Sample 28	Blood Sample 17	Blood Sample 28	Blood Sample 17	Blood Sample 28
0.43	6.05	....	60	...	23.6	....
	5.48	....	71	...	27.8	....
	4.98	....	88	...	34.8	....
	4.45	....	101	...	39.7	....
	4.11	....	168	...	66.3	....
	3.59	....	243	...	95.5	....
0.44	6.12	6.30	27	230	10.6	59.3
	5.82	5.00	40	256	15.9	65.9
	4.95	4.53	83	289	32.6	74.6
	4.48	4.07	94	331	37.1	85.4
	4.15	3.55	119	378	47.0	97.6
	3.92	....	206	...	81.0	....
	3.56	....	246	...	96.9	....
0.46	....	6.00	...	126	....	32.5
	....	5.50	...	144	....	37.0
	....	4.70	...	182	....	47.0
	....	4.00	...	295	....	76.0
	....	3.70	...	349	....	90.0
0.48	....	6.22	...	67	....	17.3
	....	5.20	...	78	....	20.0
	....	4.52	...	126	....	32.4
	....	3.98	...	248	....	63.9
	....	3.52	...	366	....	94.2

TABLE 118

PERCENTAGES OF SODIUM CHLORIDE GIVING THE SAME DEGREE OF HEMOLYSIS OF RABBIT BLOOD AT VARIOUS pH VALUES[a]

pH of NaCl Solutions	Per Cent Sodium Chloride									
6.00[b]	0.390	0.400	0.410	0.420	0.430	0.440	0.450	0.460	0.470	0.480
5.75	0.393	0.401	0.411	0.422	0.431	0.442	0.453	0.463	0.473	0.483[c]
5.50	0.396	0.402	0.412	0.424	0.432	0.444	0.455	0.465	0.475	0.485[c]
5.25	0.398	0.405	0.415	0.426	0.434	0.446	0.457	0.468	0.477	0.487[c]
5.00	0.399	0.408	0.418	0.427	0.436	0.447	0.458	0.470	0.479[c]	0.489[c]
4.75	0.405	0.411	0.422	0.431	0.441	0.453	0.464	0.475	0.485[c]	0.495[c]
4.50	0.410	0.414	0.425	0.435	0.446	0.458	0.470	0.480[c]	0.490[c]	0.500[c]
4.25	0.415	0.422	0.433	0.444	0.458	0.470	0.482	0.493[c]	0.504[c]	0.515[c]
4.00	0.420	0.430	0.440	0.453	0.470	0.482	0.494[c]	0.506[c]	0.518[c]	0.530[c]
3.50	0.475	.....	.....	.....	.....	.....	.....	.....	.....	.....

a - All of the data presented in this table are the averages of data obtained from blood samples 17 and 28.

b - The pH values of the unacidified sodium chloride solutions were all approximately 6.00.

c - These values were obtained from extrapolation of the curves from which the other values were taken directly.

TABLE 119

READINGS ON THE BECKMANN DIFFERENTIAL THERMOMETER SCALE REPRESENTING THE FREEZING POINT OF TRIPLE DISTILLED WATER

Sample	Reading	Sample	Reading
1	2.190	14	2.182
2	2.194	15	2.175
3	2.170	16	2.180
4	2.186	17	2.182
5	2.179	18	2.185
6	2.188	19	2.184
7	2.183	20	2.184
8	2.180	21	2.195
9	2.174	22	2.185
10	2.183	23	2.185
11	2.180	24	2.194
12	2.189	25	2.184
13	2.185		

Average Reading 2.184

TABLE 120

FREEZING POINT DEPRESSIONS OF 0.025 MOLAL SOLUTIONS OF VARIOUS SALTS OF GLUCONIC ACID IN DEGREES CENTIGRADE

	Salt of Gluconic Acid								
Sample	Na	K	Mg	Ca[a]	Mn(II)	Fe(II)	Co(II)	Cu(II)	Zn
1	0.081	0.086	0.132	0.050	0.144	0.110	0.116	0.066	0.122
2	0.086	0.092	0.142	0.048	0.104	0.120	0.106	0.068	0.120
3	0.094	0.086	0.132	0.052	0.100	0.114	0.122	0.071	0.124
4	0.088	0.086	0.136	0.058	0.096	0.120	0.112	0.068	0.116
5	0.090	0.088	0.108	0.046	0.096	0.126	0.118	0.080	0.116
6	0.086	0.076	0.126	0.046	0.096	0.116	0.108	0.065	0.116
7	0.088	0.086	0.136	0.045	0.114	0.101	0.104	0.072	0.118
8	0.088	0.088	0.136	0.047	0.092	0.116	0.110	0.066	0.120
9	0.086	0.074	0.126	0.058	0.100	0.106	0.120	0.065	0.119
10	0.086	0.085	0.130	0.050	0.109	0.106	0.104	0.072	0.119
Av.	0.087	0.085	0.130	0.050	0.102	0.114	0.112	0.069	0.119

[a] - Due to its low solubility, Calcium Gluconate was used as a 0.010 molal solution.

TABLE 121

_i_ VALUES OF VARIOUS SALTS OF GLUCONIC ACID CALCULATED FROM FREEZING POINT DATA OBTAINED WITH A MODIFIED BARTLEY APPARATUS

Salt	Molality of Solution	Average Freezing Point Depression[a]	_i_ Value
Sodium Gluconate	0.025	0.087	1.87
Potassium Gluconate	0.025	0.085	1.83
Magnesium Gluconate	0.025	0.130	2.80
Calcium Gluconate	0.010	0.050	2.69
Manganese(II) Gluconate	0.025	0.102	2.19
Iron(II) Gluconate	0.025	0.114	2.45
Cobalt(II) Gluconate	0.025	0.112	2.41
Copper(II) Gluconate	0.025	0.069	1.48
Zinc Gluconate	0.025	0.119	2.57

a - Data from Table 120.

TABLE 122

COMPARISON OF i VALUES FOR VARIOUS GLUCONATES OBTAINED BY THE HEMOLYTIC METHOD WITH THOSE CALCULATED FROM FREEZING POINT DATA

Gluconate	i Values from Rabbit Blood[a]	i Values from Human Blood[a]	i Values from Freezing Point Data
Sodium Gluconate	2.27	2.37	1.87
Sodium Gluconate[b]	1.99	2.07	....
Potassium Gluconate	2.26	2.28	1.83
Potassium Gluconate[b]	2.05	2.13	....
Magnesium Gluconate	2.63	4.00[c]	2.80
Magnesium Gluconate[b]	3.17	3.43	....
Calcium Gluconate	2.41	2.74	2.69
Calcium Gluconate[b]	2.99[c]	2.62	....
Manganese(II) Gluconate	2.55	2.70	2.19
Manganese(II) Gluconate[b]	2.19	2.37	....
Iron(II) Gluconate	1.27	1.61	2.45
Iron(II) Gluconate[b]	1.24	1.47	....
Cobalt(II) Gluconate	2.32	2.08	2.41
Copper(II) Gluconate	....[d]	....[d]	1.48
Zinc Gluconate	918.	582.	2.57

a - Unless otherwise indicated these i values represent an average of two blood samples.

b - Values determined in the presence of 0.2 per cent sodium chloride.

c - Average of three blood samples.

d - i values unobtainable by the hemolytic method due to the darkening of the oxyhemoglobin in the presence of copper(II) gluconate.

DISCUSSION OF RESULTS

Results of Freezing Point Determinations

In order to compare the i values obtained for the gluconates by the hemolytic method with those calculated from colligative property data, it was necessary to determine both sets of information in this laboratory; hemolytic i values for the gluconates were unavailable in the literature as were colligative property data for all the gluconates studied except for iron(II) and calcium.

Since gluconic acid is capable of forming water soluble complexes with certain metallic ions, it was recognized that freezing point data would be helpful in evaluating the results of the hemolytic experiments. The i values calculated from the freezing point depressions obtained in the experiments with sodium, potassium, magnesium, and calcium (1.87, 1.83, 2.80, and 2.69 respectively) were similar to the i values of salts of these metals with inorganic acids; therefore it was assumed that there was no association of these cations by gluconic acid.

Cobalt(II), iron(II), and zinc gluconates appeared to be slightly associated as their i values were 2.41, 2.45, and 2.57 respectively. Manganese(II) gluconate seemed to be more strongly associated as it had an i value of 2.19 whereas copper(II) gluconate exhibited an extremely low i value of 1.48. This seemed to support the work of others (53) who have reported that gluconic acid formed very strong complexes with most of the transition elements.

It should be remembered, however, that any excess of gluconic acid in the samples would yield data which would be interpreted in terms indicating association and low _i_ values. Also, in relating these _i_ values to those obtained by the hemolytic method, it must be borne in mind that the addition of the blood components may alter the degree of association of the metallic gluconates. The original pH values of the gluconate solutions did not change appreciably after the addition of blood (_vid._ Table 116) so that a variation in association due to pH change was assumed to be negligible. Indeed, the working temperatures of the two methods have to be considered in comparing the _i_ values obtained by the different methods; the hemolytic experiments were all carried out at $37^{\circ} \pm 1^{\circ}$.

Variability of Blood

The osmotic fragility of erythrocytes varied from animal to animal and, indeed, from day to day in the same animal; employing blood from the same donor, daily variations were observed in the degree of hemolysis occurring in a given concentration of sodium chloride solution. This variability was compensated for during each determination by ascertaining the degree of hemolysis occurring in both the reference sodium chloride solutions and the gluconate solutions.

Human blood generally produced greater colorimetric readings for 100 per cent hemolysis than did rabbit blood. Negro blood employed in two experiments early in this study (Table 5, Blood Samples 51 and 52) failed to become 60 per cent hemolyzed in 0.32 per cent sodium chloride solutions, a salt concentration which in most instances seemed to completely lake erythrocytes from white donors. Eight additional blood samples from Negro volunteers were obtained and the osmotic fragility of the erythrocytes determined in various concentrations of sodium chloride (Table 5, Blood Samples 60 - 67). Although two of the ten Negro blood samples studied exhibited greater than 90 per cent hemolysis in 0.32 per cent sodium chloride solutions, seven samples showed less than 80 per cent and three samples less than 60 per cent hemolysis. An average of 74.7 per cent hemolysis was shown by the ten Negro blood samples in 0.32 per cent sodium chloride; the samples of Caucasian donors' blood employed in this study showed an average of 97.0 per cent hemolysis in 0.32 per cent sodium chloride solutions. Blood from Caucasian donors showed, on the average, 50 per cent hemolysis in sodium chloride solutions of 0.381 per cent concentration; the same degree of hemolysis did not occur in Negro blood until the sodium chloride concentration was decreased, on the average, to 0.342 per cent. All of the Caucasian blood samples investigated hemolyzed to a certain degree in 0.42 per cent sodium chloride concentration whereas only four of the Negro blood samples exhibited hemolysis in this salt concentration.

Diseases of the blood and other pathological conditions have long been known to cause a variation in the osmotic fragility of erythrocytes (166). Sickle cell anemia, a disease occurring almost entirely in the Negro race, is characterized by sickle-shaped red blood cells and by an increase in the resistance of these cells towards hypotonic saline.

In this present study, the Negro blood samples were all examined for evidence of sickle cells within two hours after the blood was drawn.

An ordinary laboratory microscope was used, employing a 430X magnification; there was no evidence of sickling observed in the blood samples.

Turner et al. (167) in a study employing blood from 94 Negroes found that in 22 per cent of the cases complete hemolysis did not occur until the sodium chloride concentration was reduced to 0.28 per cent. In 14.8 per cent of the cases, salt concentrations above 0.36 per cent were adequate in preventing hemolysis; by increasing the proportion of salt to 0.40 per cent, hemolysis was not observed in 72.2 per cent of the samples.

By studying the data of the present work and that of Turner et al. (167) it appears that the "normal average values of osmotic fragility of human blood" in sodium chloride solutions, presented in most hematology texts, refer to blood of Caucasians only.

The Effect of Hydrogen Ion Concentration on Hemolysis

The experiment designed to determine the maximum acidity that a solution may have without altering the color of oxyhemoglobin showed that solutions more acidic than pH 4 darkened the color of oxyhemoglobin. Brown colored hematin has been said to result from a strongly acid hemoglobin solution with complete transformation taking place within twenty-five minutes at pH 3.8 (163).

The experiment carried out to determine the effect of increasing the hydrogen ion concentration on hemolysis indicated that in a given concentration of sodium chloride solution an increase in the acidity caused an increase in hemolysis; vid., Tables 117 and 118. For example, at pH 6.00, a 0.390 per cent solution of sodium chloride was sufficient to protect a certain proportion of the erythrocytes from hemolyzing; at pH 5.00, however, a sodium chloride concentration of 0.399 per cent was required to protect the same proportion of red blood cells. By utilizing the data on Table 118, a portion of the hemolysis occurring in gluconate solutions more acidic than the reference sodium chloride solutions was attributed to the hydrogen ion concentration and an attempt was made to correct the i values accordingly. Iron(II) gluconate, for example, caused 50 per cent hemolysis of blood sample 22 (vid. Table 82) at a concentration of 5.19 per cent while the same degree of hemolysis occurred in a 0.477 per cent sodium chloride solution. The pH values of the iron(II) gluconate solutions were approximately 4.55 (vid. Table 116). By the utilization of Table 118, it was found that at pH 4.55 a sodium chloride solution of 0.496 per cent was required to protect the same proportion of cells as a salt solution of 0.477 per cent concentration at pH 6.00. Thus the i value of 1.31 calculated for iron(II) gluconate was in error as a result of hemolysis due to the hydrogen ion concentration. A recalculation of this i value, by replacing the per cent of sodium chloride found to protect 50 per cent of the erythrocytes with the salt concentration which protected the same proportion of cells at the pH of the iron(II) gluconate solutions, gave an i value of 1.36. Because of some uncertainties, as the actual pH at the cell membrane, and since the changes in the i values appeared to be quite small the i values originally obtained for the gluconates were reported without an adjustment for the acidity of the solutions. It was recognized, however, that the correction for the i values might represent a minimum one since the gluconate solutions resisted a change in pH upon the addition of blood (vid. Table 116) whereas the acidified sodium chloride solutions were almost immediately brought to a pH value near the pH of the blood and the action of the hydrogen ions was probably only momentary.

Results of Blood Protein Precipitation Experiments

Sodium, potassium, calcium, magnesium, manganese(II), and cobalt(II) gluconates did not seem to precipitate any of the serum proteins of either human or rabbit blood. Iron(II) gluconate solutions, if over a couple of hours old, became hazy upon the addition of human or rabbit blood serum. After standing in a water bath at 37° for forty-five minutes, and centrifugation, the precipitates appeared tan colored and rather bulky. During its addition, the serum did not appear to coagulate as it touched the surface of the iron(II) gluconate solution; serum coagulation was instantaneous in both copper(II) and zinc gluconate solutions. Fresh solutions of iron(II) gluconate remained clear on the addition of blood serum and no precipitation was observed after the standing period and centrifugation. The copper(II) gluconate-serum protein precipitate redissolved on standing (and instantaneously on agitation) and was milk-white in color. The addition of alkali to the copper(II) gluconate-serum mixture, containing redissolved serum, resulted in a voluminous blue-white precipitate. The zinc gluconate-serum protein precipitate was milk-white in color, voluminous, and appeared to be stable.

No precipitation was noticed when oxyhemoglobin solutions, prepared from human and rabbit erythrocytes, were mixed with separate solutions of sodium, potassium, calcium, magnesium, manganese(II), cobalt(II), and copper(II) gluconates. Copper(II) gluconate solutions did, however, turn the oxyhemoglobins brown. Iron(II) gluconate solutions, if not fresh, and both human and rabbit oxyhemoglobin solutions combined to form brown colored sediments. Zinc gluconate precipitated a portion of the oxyhemoglobins from both human and rabbit bloods, but less effectively than when a proportion of serum was present. Also, when serum and oxyhemoglobin were separately added to a zinc gluconate solution, the precipitation was less complete than when the two proteins were mixed before being added.

Additional discussion will be presented under the headings of the individual gluconates.

Results with Sodium and Potassium Gluconates

Employing the hemolytic method, sodium and potassium gluconates gave higher i values than would be expected for compounds yielding two ions. Freezing point information indicated that each of these gluconates acted normally, i.e., as slightly less than two particles; vid. Table 121.

Sodium gluconate exhibited an average i value of 2.27 using rabbit blood and 2.37 using human blood. These values were lowered to 1.99 with rabbit blood and 2.07 with human blood when determined in the presence of 0.2 per cent sodium chloride.

Potassium gluconate gave an average i value of 2.26 using rabbit blood and 2.28 using human blood. These values were lowered to 2.05 with rabbit blood and 2.13 with human blood when the determinations were conducted in the presence of 0.2 per cent sodium chloride; vit. Tables

114 and 115.

In solutions of some substances, erythrocytes may lose electrolyte from within the cell, thus causing a decrease in the internal osmotic pressure and a corresponding increase in resistance to hemolysis (2, 168, 169). The exosmosis of erythrocytes in such solutions can be corrected in some cases by the addition of small proportions of an electrolyte, e.g., sodium chloride (2, 169).

The high hemolytic i values obtained for sodium and potassium gluconates may be attributed to exosmosis of electrolyte from within the erythrocytes. The lowering of the i values upon the addition of sodium chloride may be due to the partial restoration of the environment normal to the red blood cells

Results with Calcium and Magnesium Gluconates

The hemolytic i values for calcium gluconate, calculated from data of concentrations causing 25, 50, and 75 per cent hemolysis of human erythrocytes, increased by approximately 0.2 (2.54 to 2.72) going from 25 to 50 per cent hemolysis and another 0.2 (2.72 to 2.95) going from 50 to 75 per cent hemolysis. A similar observation was made by previous investigators of calcium acetate, calcium gluconate, and calcium levulinate (6, 122). This steady increase did not occur when the i values for calcium gluconate were determined in the presence of 0.2 per cent sodium chloride; vid. Table 115.

Hemolytic i values for calcium gluconate obtained with rabbit blood increased by approximately 0.1 (2.32 to 2.40) going from 25 to 50 per cent hemolysis and by another 0.1 (2.40 to 2.50) going from 50 to

75 per cent hemolysis. Increases of similar magnitude were noted when the determinations were made in the presence of 0.2 per cent sodium chloride.

The presence of 0.2 per cent sodium chloride seemed to cause an increase in the i values for calcium gluconate using rabbit blood and a decrease in the i values when human blood was used. In this respect, magnesium gluconate acted similarly to calcium gluconate.

The i values obtained for magnesium gluconate were higher in every analogous case than those obtained for calcium gluconate. Using human blood, an average i value of 4.00 was obtained for magnesium gluconate which was lowered to 3.43 when determined in the presence of 0.2 per cent sodium chloride. These i values are much beyond that calculated from freezing point data, viz. 2.80.

Using blood of the rabbit, the i values for magnesium gluconate increased from 2.63 to 3.17 when the determinations were conducted in the presence of 0.2 per cent sodium chloride.

According to Ponder (170), there is considerable exchange of internal potassium for alkaline earth metals in human cells under certain conditions. All of the alkaline earths make the cells more easily broken by mechanical stress, calcium being particularly effective in this respect (171). Davson and Danielli (172) noted the difference in the response of the cat red blood cell membrane to sodium and potassium permeability in the presence of alkaline earths; they showed that tonicity changes were responsible for only a portion of their observed results. Alkaline earth ions were reported to thicken the erythrocyte membrane by forming insoluble salts with the phosphatide acids present in the cell wall (130).

Under normal conditions, calcium does not cross the red cell membrane (129). The effects of calcium could be many, including: a) participation in the competition for water, b) solidifying effects, and c) combinations with proteins (173, 174). Although evidence of protein combinations with calcium and magnesium was not observed in this laboratory, the interaction of these alkaline earths with surface proteins of the erythrocyte could conceivably affect the penetration of ions into and out of the cell.

Danielli (175), in a discussion of the action of narcotics (defined in terms of inhibition of various activities), mentioned that there is a good deal of evidence that substances such as magnesium act primarily on the cell surface, in some way modifying the excitability of the cell. He explained that although there is no conclusive evidence, it is best to consider these actions as being restricted to cell surfaces.

It is not unlikely that many of the aforementioned actions of the alkaline earths could have contributed to the results obtained in this study of calcium and magnesium gluconates.

Results with Cobalt(II) and Manganese(II) Gluconate

Cobalt(II) and manganese(II) gluconates each formed colored solutions in triple distilled water. The procedure on page 32 was carried out to determine if the color of either of these gluconate solutions was additive with the color of oxyhemoglobin solution. Manganese(II) gluconate solutions did form an additive color and blank readings were employed according to the aforementioned method. Cobalt(II) gluconate solutions exhibited colors that were not additive with that of oxyhemoglobin solution; thus the degree of hemolysis of erythrocytes in solutions of cobalt(II) gluconate was determined by the alternate method for colored solutions presented on pages 32 to 34.

Cobalt(II) gluconate gave i values of 2.32 with rabbit erythrocytes and 2.08 with human erythrocytes; freezing point information indicated an i value of 2.41. The lower i values obtained by the hemolytic method may be attributed, in part, to the acidity of the cobalt solutions (about pH 5). Such acidity could cause hemolysis, independent of the osmotic pressure of the solution; this additional hemolysis would result in data from which low i values would be calculated.

Manganese(II) gluconate gave i values of 2.55 with rabbit erythrocytes and 2.70 with human erythrocytes. In the presence of 0.2 per cent sodium chloride, the i values with each blood type were lowered by approximately the same amount, i.e., 0.33 using human blood and 0.36 using rabbit blood. These lower i values more closely resembled the i value obtained for manganese(II) gluconate by the freezing point depression method, viz. 2.19.

There was no evidence of protein precipitation by either cobalt(II) or manganese(II) gluconate solutions.

Results with Iron(II) Gluconate

Employing the hemolytic method, iron(II) gluconate gave i values of 1.27 with rabbit blood and 1.61 with human blood. These values were lowered to 1.24 with rabbit blood and 1.47 with human blood when the determinations were carried out in the presence of 0.2 per cent sodium chloride. These values are all lower than what would be expected for a compound yielding three ions; an _i_ value of 2.45 was obtained for iron(II) gluconate by the freezing point depression method.

In triple distilled water, iron(II) gluconate formed colored solutions that were found to be additive with the color of oxyhemoglobin solution; therefore blank readings were employed in accordance with the procedure presented on pages 32 to 34.

Throughout the course of this investigation, there was a tendency to be doubtful of the stability of the iron(II) gluconate solutions. Stone (176) stated that though iron(II) gluconate is highly stable in the solid state, its solutions are quite the opposite. They are prone to oxidation, with iron(II) changing to iron(III); the rate of transformation being dependent on the pH, temperature, degree of aeration, presence of oxidizing or reducing agents, etc. Oxidation causes a darkening of the solutions, a characteristic which would make colorimetric assays unreliable. An iron(III) compound of unknown composition has been found to precipitate from aqueous solutions of iron(II) gluconate as a result of a photochemical reaction.

Although the iron(II) gluconate solutions were prepared just prior to being used, precipitates were often noticed in the colorimeter tubes during the course of the determinations. These brown colored precipitates generally occurred in the iron(II) gluconate-blood mixtures containing the lower proportions of the iron salt. If a portion of the iron(II) gluconate was precipitated as an oxidized form, fewer particles would be in solution to aid in the prevention of osmotic hemolysis; the resulting data would indicate less protection and thus, a lower i value.

Oftentimes, the supernatant solutions seemed to contain colloidally suspended particles that could not be centrifuged down within the prescribed time; the possibility of laking the unhemolyzed cells by increasing the speed and/or time of centrifugation negated the thought of altering the general method.

According to several investigators (77,78, 176) iron(II) gluconate does not precipitate proteins, a property that makes it valuable in parenteral administration. Although this present work has indicated that human and rabbit sera and oxyhemoglobins were not visibly affected by fresh iron(II) gluconate solutions, they were affected by solutions not freshly prepared.

Human plasma contains a metal-combining globulin, beta-globulin, that has a high affinity for metal ions, particularly iron, copper, and zinc (177). Beta-globulins are not always saturated to capacity with iron, and when such is the case iron has been shown to be able to more rapidly penetrate the red cell membrane in vitro (178).

The binding of iron(II) gluconate by plasma proteins, with or without precipitation, would decrease the number of available particles in solution and the i values obtained would be lower than normal.

The pH of the iron(II) gluconate solutions was about 4.55 and remained approximately the same during the course of each determination. Hemolysis due to acidity would produce data from which low i values would result.

Results with Copper(II) Gluconate

Copper(II) gluconate in concentrations from 1 to 10 per cent seemed to cause irreversible denaturation of blood; hemoglobin released from laked erythrocytes turned brown as did the unhemolyzed red blood cells. It has been reported (179) that copper sulfate, chloride, nitrate, and acetate have the ability to precipitate erythrocytes.

The pH of the copper(II) gluconate solutions employed in this study was approximately 4, this pH remaining fairly constant throughout the duration of the experiments. Bloch and Oelsner (163) have reported that at pH 3.0 hemoglobin is instantaneously converted to brown colored hematin, at pH 3.8 it is converted within twenty-five minutes, and at pH 4.6 the formation of hematin is incomplete in twenty-four hours. They stated that the rate of conversion is directly proportional to the hydrogen ion concentration.

According to Meneghetti (133), the activity of copper as a hemolytic agent is superior to that of many other metals. Lambin and co-workers (136, 137) reported that many copper salts had acted similarly in their hemolysis experiments; erythrocytes were hemolyzed in concentrations greater than 1.3×10^{-5} mole/liter and were agglutinated in concentrations of from 1×10^{-2} to 1×10^{-5} mole/liter.

In the tests run to determine the protein precipitating action of copper(II) gluconate, it was found that both rabbit and human serum proteins were to a certain extent precipitated by this gluconate. These precipitates dissolved on standing or on gentle agitation. Cohn (180) reported that copper reacts with serum albumin to a small extent.

Ressler et al. (181), in a study with human serum at 37°, found that copper was more effective than nickel, zinc, cobalt, iron, and manganese (listed in order of decreasing effectiveness) in causing turbidity with serum. Isolation of these turbid substances showed that they consisted primarily of albumin and gamma-globulin, with the cations combined in definite proportions.

Due to the darkening of the blood cells and of oxyhemoglobin, i values for copper(II) gluconate were unobtainable by the hemolytic method. The erythrocytes were not prevented from turning brown when experiments were carried out in the presence of 0.6 per cent sodium chloride. Freezing point data indicated that copper(II) gluconate is highly associated, giving an i value of 1.48 instead of the usual 2.70 for a compound yielding three ions.

Results with Zinc Gluconate

Extremely high i values were calculated for zinc gluconate from data obtained using the hemolytic method. The values for i at concentrations causing 25, 50, and 75 per cent hemolysis of rabbit erythrocytes were 793, 933, and 1027 respectively, whereas concentrations causing 50 and 75 per cent hemolysis of human erythrocytes showed i values of 499 and 666 respectively. Values of i at 25 per cent hemolysis of human erythrocytes were unobtainable.

Throughout the course of this investigation, unusual behavior was noticed between blood and zinc gluconate solutions. Blood appeared to agglutinate as it was added to colorimeter tubes containing solutions of the zinc salt; at the same time, a milk-white formation presented itself around the blood. Before inverting the tubes (to mix their contents) a layer of clear oxyhemoglobin solution was observed at the bottom of some of the tubes; after inverting the tubes, the contents became uniformly opaque. A sediment was observed at the bottom of each tube after centrifugation which was of a volume greater than could be attributed to the small proportion of blood present. The substance was a reddish color (resembling cosmetic rouge) and was amorphous under the microscope. The degree of hemolysis was determined in the usual manner and $\underline{i}$ values calculated according to the general methods.

Rawlinson (147) described a zinc-oxyhemoglobin compound precipitated from a solution of rabbit blood pigment by zinc sulfate. The substance was of a reddish color and soluble in dilute ammonia and in dilute acetic acid solutions.

Gurd and Goodman (182) stated that the property of protein that is most obviously affected by combinations with metallic cations is solubility. To affect the solubility of protein by mechanisms other than "salting in" or "salting out," the metallic ion must be bound to the protein. To these workers, one of the most striking properties of the metal-protein precipitates studied was the large volume that they occupied. They explained that the bulkiness indicated that under certain conditions precipitation has more the form of gelation than of the separation of a precipitate from a supernatant solution.

Oxyhemoglobin solution, prepared in this laboratory by laking washed rabbit erythrocytes in triple distilled water, was partially precipitated when placed in contact with a 1 per cent solution of zinc gluconate. The precipitate appeared to be soluble below pH 5.5 and above pH 10.0; it appeared to be least soluble at around the neutral point. Oxyhemoglobin solutions prepared from washed human erythrocytes acted in a similar manner.

Human and rabbit blood sera, separated from the defibrinated bloods by centrifugation, were each precipitated in the presence of zinc gluconate. The white, curdy precipitates were soluble below pH 5.0.

A detailed study of the competition between zinc and hydrogen ions for common sites in human serum albumin led Gurd and Goodman (182) to conclude that the imidazole groups of the 16 histidine residues were the principal sites of binding of zinc ion by this protein.

Gurd (183) stated that the effect of zinc ions on the solubility of serum albumin is greatest in the pH range of about 6.5 to 7.0. He said that both gamma-globulin and beta-lipoprotein can be precipitated in the presence of zinc acetate. In these cases, gamma-globulin aided in the precipitation of the beta-lipoprotein. Gurd found that for all of the plasma proteins that he studied, the minimal solubility in the presence of zinc ions at 0° occurred at pH 7.0. On the alkaline side of neutrality, the formation of mixed complexes of zinc ions with hydroxyl ions and imidazole molecules was mentioned as being quite likely. The presence of an anion with a strong tendency to form complexes has, generally, reduced the affinity of the metallic ion for the groups in the protein. In certain cases, an anion may compete so effectively that only the groups in the protein having the highest order of affinity are able to bind the metallic ion.

When blood was added to a solution containing 1 per cent of zinc gluconate and 0.85 per cent of sodium chloride, the cells settled to the bottom of the tube and failed to hemolyze after forty-five minutes at 37°. After centrifugation, the cells were bound together at the bottom of the tube and failed to hemolyze after the supernatant solution of zinc gluconate-sodium chloride-serum was decanted and replaced with triple distilled water. Inversion of the tube caused the cells to pass through the water, still bound together and resembling a "pancake."

Gibson (184) reported that red cells take up zinc very readily; whether the zinc enters the cell or is bound to the membrane is not known. It has been shown, however, that the zinc cannot be removed by washing the cells in isotonic saline.

It appears obvious that the high $\underline{i}$ values obtained for zinc gluconate in this work and those $\underline{i}$ values obtained for zinc sulfate and zinc acetate in other works (4, 5) have been due to a shortcoming of the hemolytic method, i.e., failing to provide a means of determining that amount of liberated oxyhemoglobin that has been altered in such a manner to cause it to be unaccounted for by the photoelectric colorimeter. A soluble complex between an ion and oxyhemoglobin may change the proportion of light absorbed by the solution and, indeed, may cause error in the photoelectric assay without any noticeable warning, such as precipitation or darkening.

SUMMARY

The adjustment of solutions intended for intravenous administration to the same tonicity as blood is of prime importance; the advantages of employing the hemolytic method in the preparation of such solutions have been emphasized by Husa and co-workers (1, 2, 3, 4, 5, 6, 7, 8). It is now recognized that, when dealing with blood, the osmotic effect of a chemical substance depends not only on its concentration but also on whether or not it penetrates the erythrocyte membrane or affects its properties; thus solutions calculated to be isosmotic with blood by physicochemical data are not necessarily isotonic to the red blood cell.

The purpose of the present investigation was to determine _i_ values for various salts of gluconic acid by both the freezing point depression and hemolytic methods. The effect of the hydrogen ion concentration on hemolysis was determined and a study made of the action of some gluconates in precipitating blood proteins. A method was devised for determining the degree of hemolysis of erythrocytes suspended in colored solutions. An explanation of the unusually high hemolytic _i_ values obtained for some zinc salts by previous workers was offered. A comparison was made of the osmotic fragility of erythrocytes obtained from Caucasian and Negro donors; red blood cells from Negro donors were found to be, on the average, more resistant to osmotic hemolysis.

Using the hemolytic method, sodium and potassium gluconates gave _i_ values which were approximately 25 per cent higher than those obtained

by the freezing point depression method; this difference was only half as great when the hemolytic determinations were conducted in the presence of 0.2 per cent sodium chloride.

Magnesium and calcium gluconates gave higher i values by the hemolytic method than by the freezing point depression method when human erythrocytes were employed and lower ones when rabbit erythrocytes were used. The addition of 0.2 per cent sodium chloride raised the hemolytic i values of these salts when rabbit erythrocytes were used and lowered the values when human blood was used.

Employing the freezing point depression method, manganese(II) gluconate gave an i value that was lower than normal for a compound yielding three ions. Hemolytic determinations conducted in the presence of 0.2 per cent sodium chloride gave similarly low i values whereas hemolytic i values obtained in the absence of additional electrolyte were moderately higher.

Iron(II) gluconate solutions appeared to be unstable, and gave i values by the hemolytic method which were lower than what would be expected for a compound yielding three ions. The addition of 0.2 per cent sodium chloride failed to bring the i values closer to normal.

Cobalt(II) gluconate gave lower than normal i values by the hemolytic method and by the freezing point depression method, thus indicating association of cobalt(II) gluconate in solution.

Hemolytic i values for copper(II) gluconate were unobtainable due to a darkening of the oxyhemoglobin and the denaturation of the red blood cells; freezing point data indicated a high degree of association in copper(II) gluconate solutions.

Zinc gluconate exhibited extremely high hemolytic _i_ values due to the partial precipitation of the oxyhemoglobin liberated from laked erythrocytes making the colorimetric readings of the supernatant liquid misleading.

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BIOGRAPHICAL ITEMS

Howard Carl Ansel was born in Cleveland, Ohio on October 18, 1933. He was educated in the public schools of that city and of Phoenix, Arizona, and was graduated from John Adams High School in Cleveland in June, 1951.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of the committee. It was submitted to the Dean of the College of Pharmacy and to the Graduate Council and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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